1 agggagaggc agtgaccatg aaggctgtgc tgcttgcccc gttgatggca

51 ggcttggccc tgcagccagg cactgccctg ctgtgctact cctgcaaagc

101 ccaggtgagc aacgaggact gcctgcaggt ggagaactgc acccagctgg

151 gggagcagtg ctggaccgcg cgcatccgcg cagttggcct cctgaccgtc

201 atcagcaaag gctgcagctt gaactgcgtg gatgactcac aggactacta

251 cgtgggcaag aagaacatca cgtgctgtga caccgacttg tgcaacgcca

301 gcggggcccа tgccctgcag ccggctgccg ccatccttgc gctgctccct

351 gcactcggcc tgctgctctg gggacccggc cagctatagg ctctgggggg

401 ccccgctgca gcccacactg ggtgtggtgc cccaggcctt tgtgccactc

451 ctcacagaac ctggcccagt gggagcctgt cctggttcct gaggcacatc

501 ctaacgcaag tttgaccatg tatgtttgca ccccttttcc ccnaaccctg

551 accttcccat gggccttttc caggattccn accnggcaga tcagttttag

601 tganacanat ccgcntgcag atggcccctc caaccnttth tgttgntgtt

651 tccatggccc agcatttttcc acccttaacc ctgtgttcag gcacttnttc

701 ccccaggaag ccttccctgc ccaccccatt tatgaattga gccaggtttg

751 gtccgtggtg tccccccgcac ccagcagggg acaggcaatc aggagggccc

801 agtaaaggct gagatgaagt ggactgagta gaactggagg acaagagttg

851 acgtgagttc ctgggagttt ccagagatgg ggcctggagg cctggaggaa

901 ggggccaggc ctcacatttg tggggttccc gaatggcagc ctgagcacag

951 cgtaggcccc taataaacac ctgttggata agccaaaaaa aaaaaaaa

FIGURE 1A

MKAVLLALLMAGLALQPGTALLCYSCKAQVSNEDCLQV

ENCTQLGEQCWTARIRAVGLLTVISKGCSLNCVDDS

QDYYVGKKNITCCDTDLCNASGAHALQPAAAILALLPAL

GLLLWGPGQL

FIGURE 1B

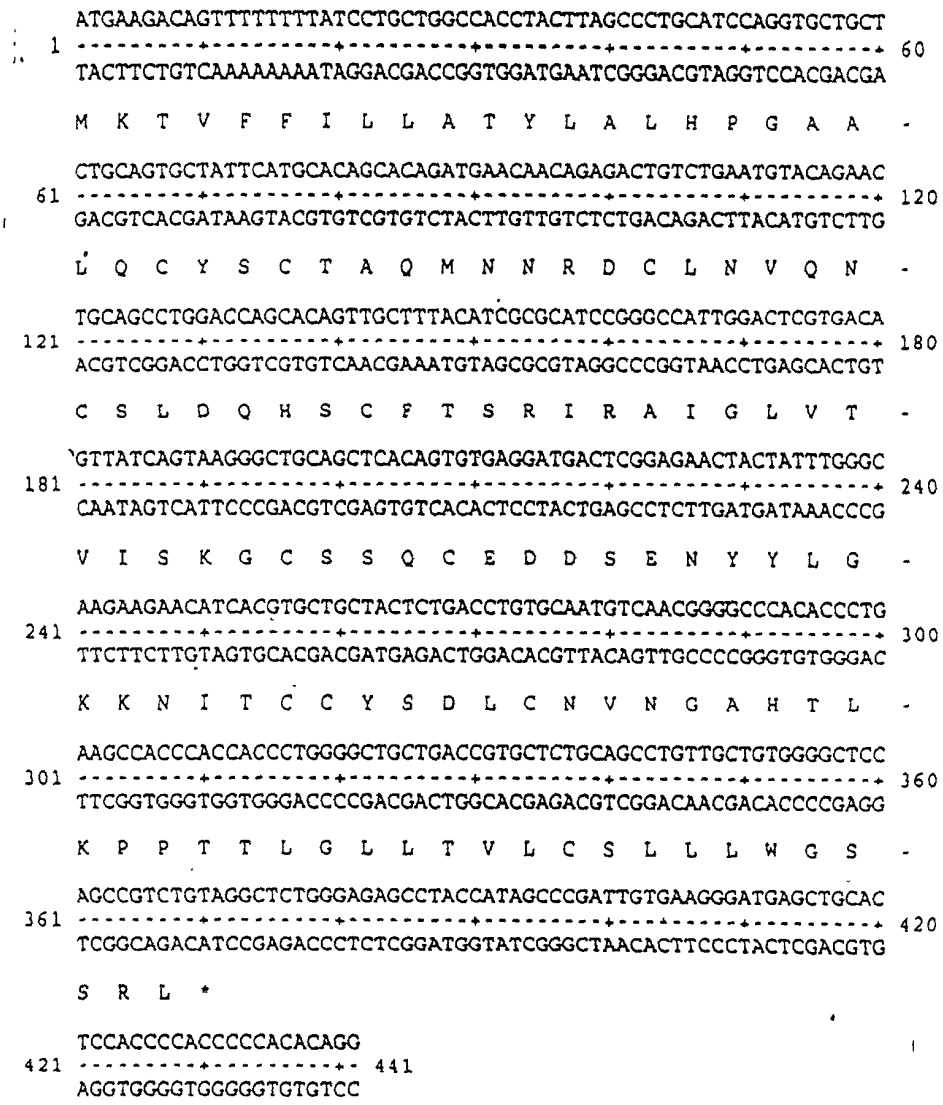


FIGURE 2

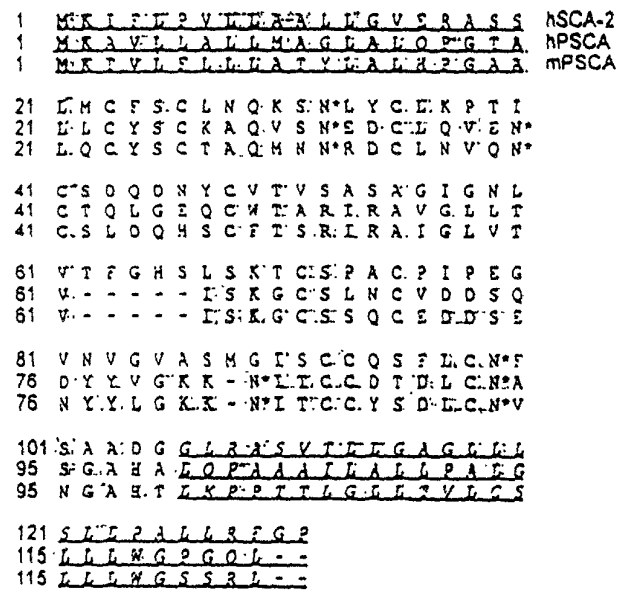


FIGURE 3

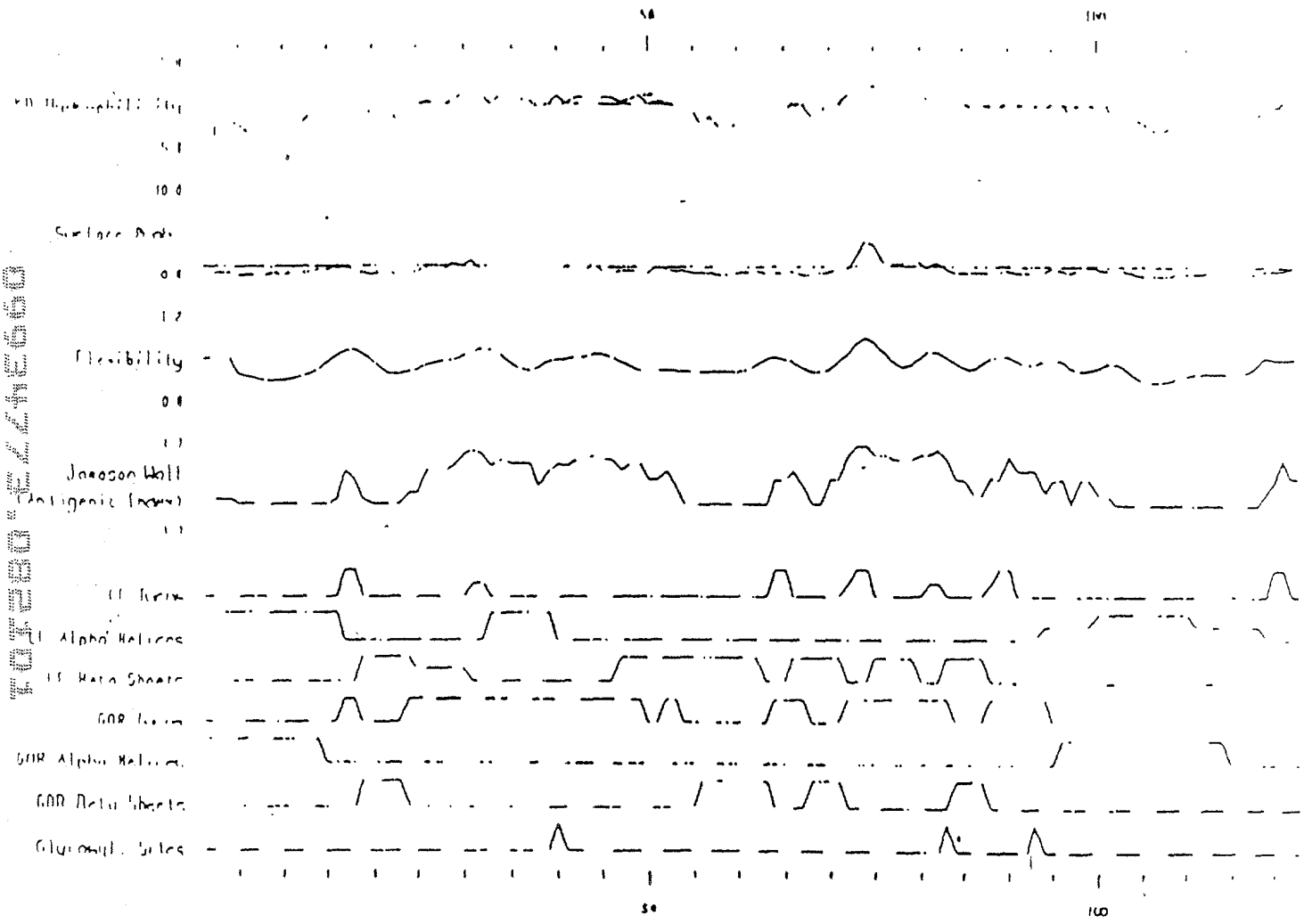


FIGURE 4

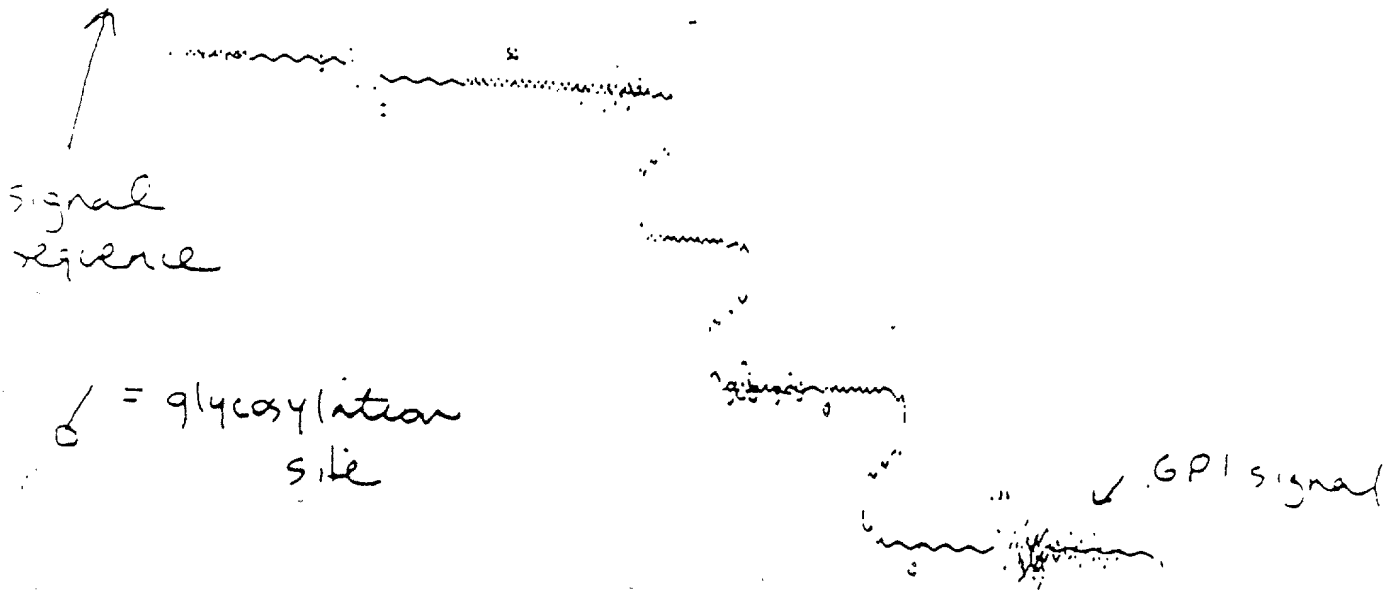


FIGURE 5

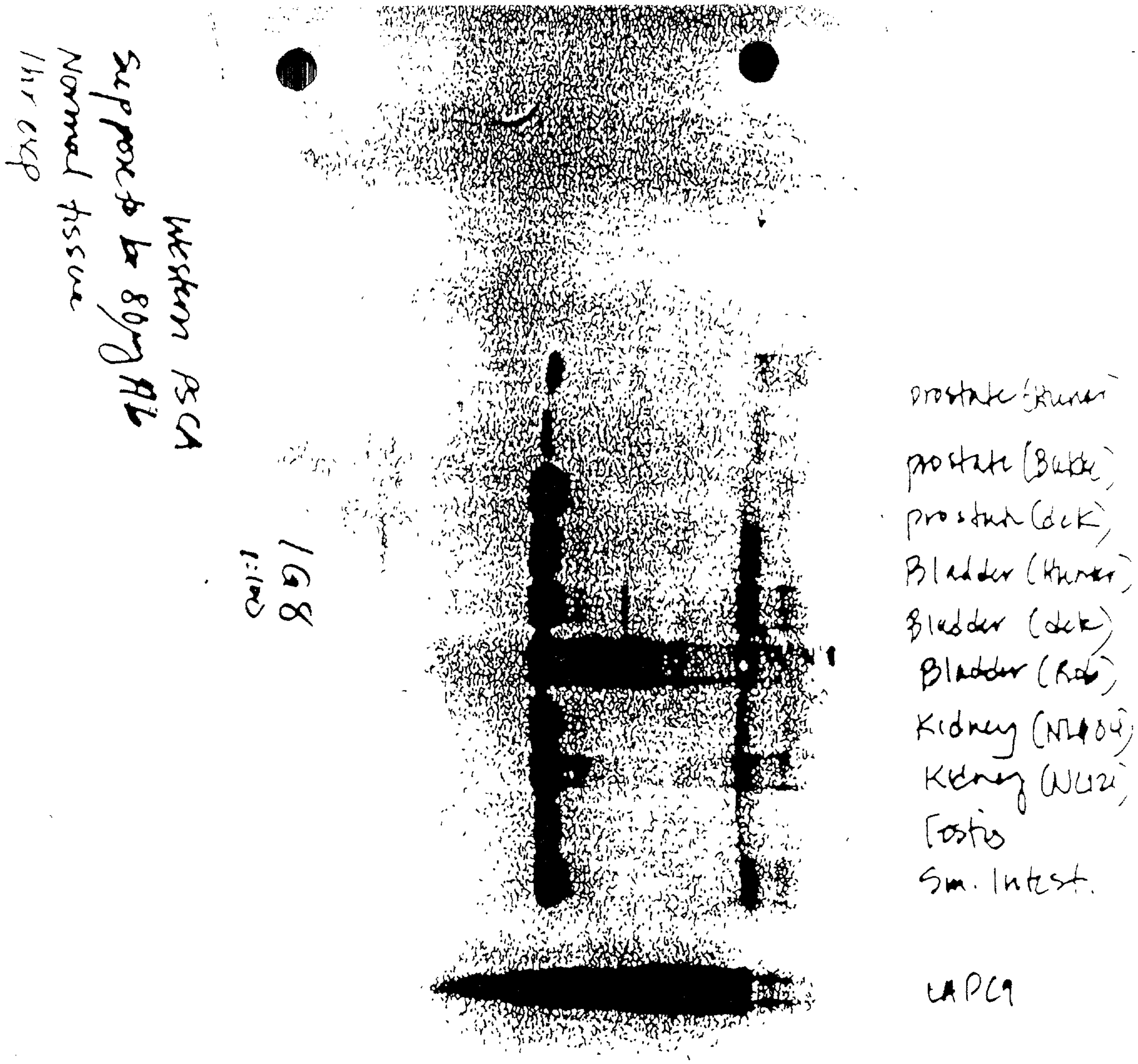

Western
Normal tissue
Bladder
Bladder
Bladder
Kidney
Testis
Sm. Intest.
LAPC9

FIGURE 6

FIG. 7A

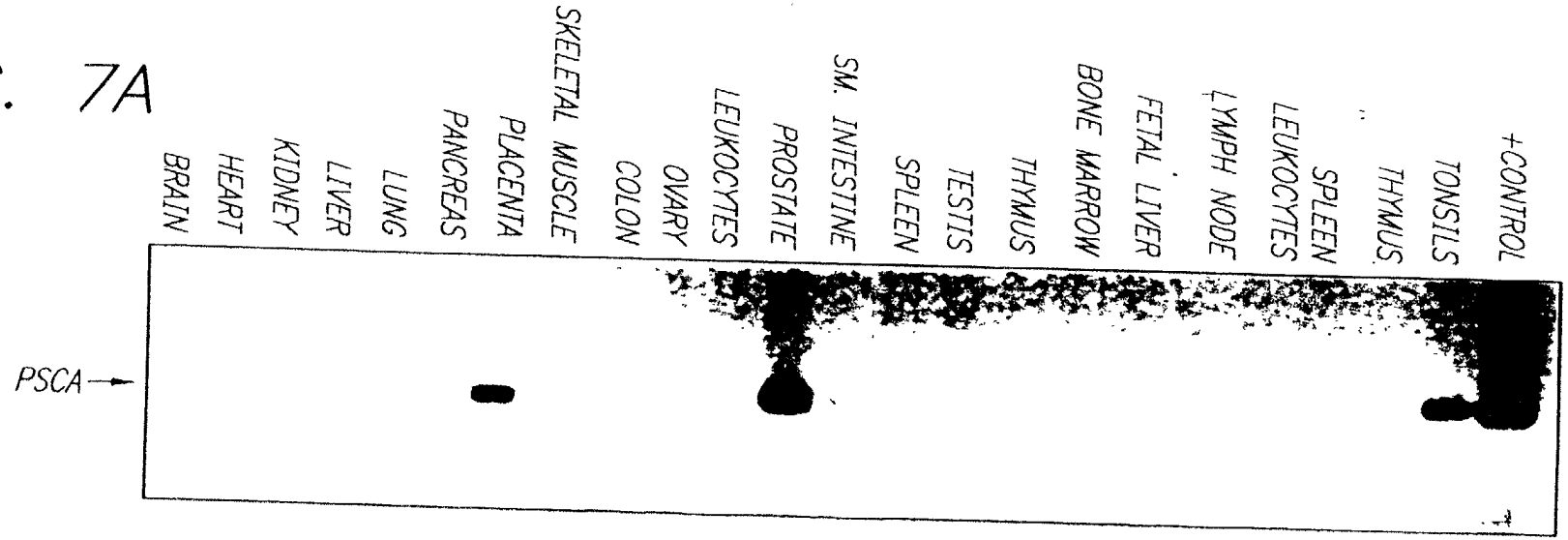
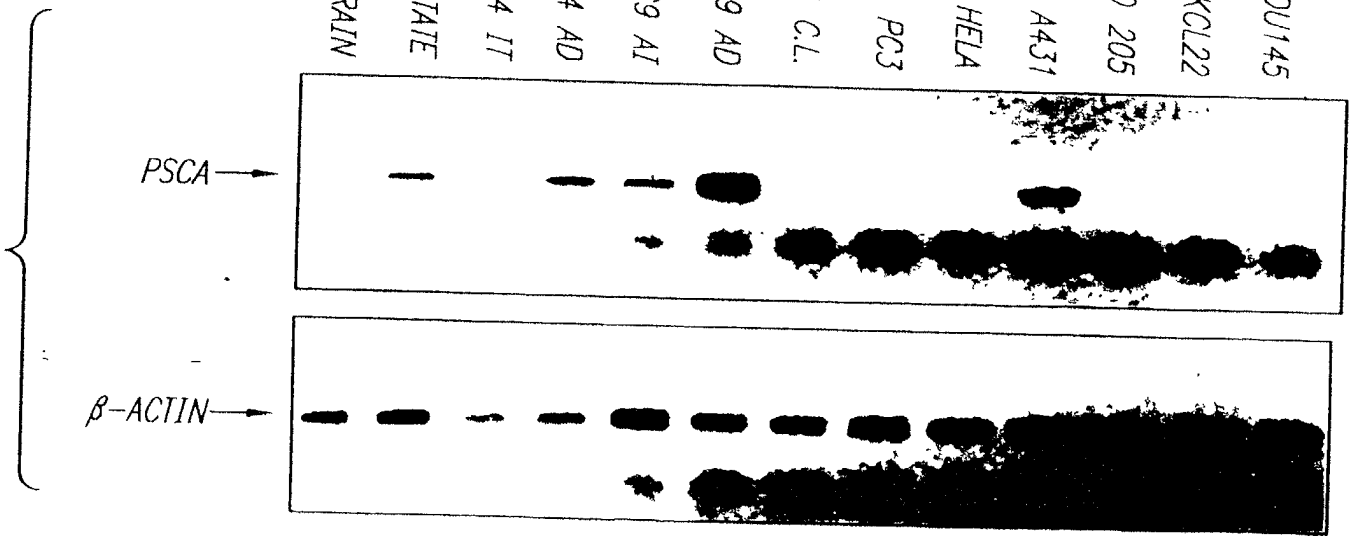

+CONTROL
TONSILS
THYMUS
SPLEEN
LEUKOCYTES
LYMPH NODE
FETAL LIVER
BONE MARROW
THYMUS
TESTIS
SPLEEN
SM. INTESTINE
PROSTATE
LEUKOCYTES
OVARY
COLON
SKELETAL MUSCLE
PLACENTA
PANCREAS
LUNG
LIVER
KIDNEY
HEART
BRAIN
PSCA

FIG. 7B


DU145
KCL22
COLO 205
A431
HELA
PC3
LAPC4 C.L.
LAPC9 AD
LAPC9 AI
LAPC4 AD
LAPC4 IT
PROSTATE
BRAIN
PSCA
β-ACTIN

legend: untranslated region of PSCA

translated region of PSCA

Ly-6 Thy-1

FIG. 8A

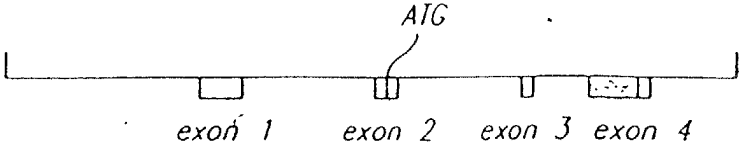


FIG. 8B

FIGURE 8

murine PSCA

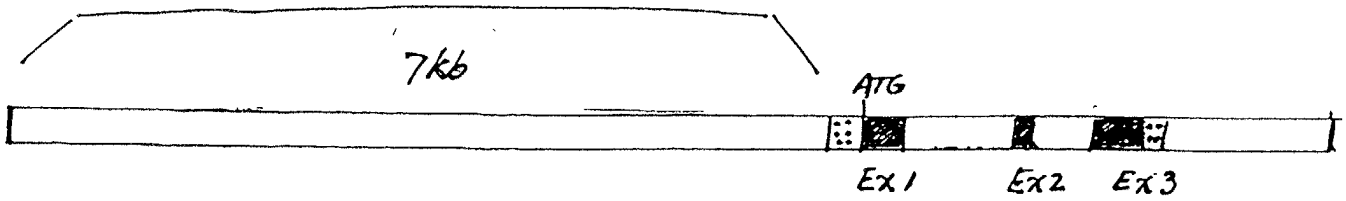
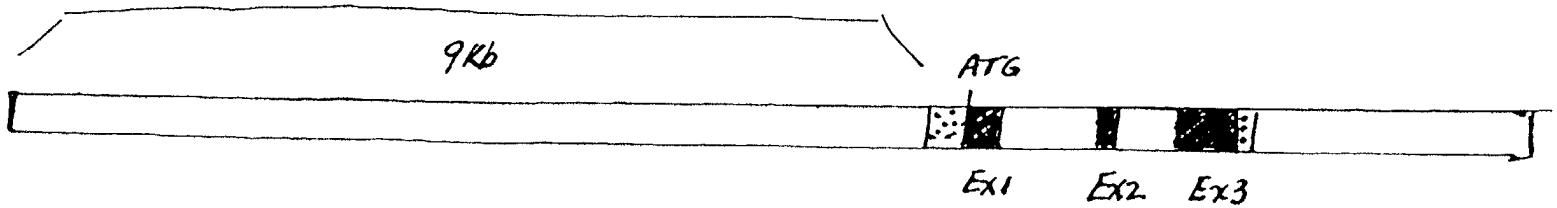


FIG. 8C

human PSCA



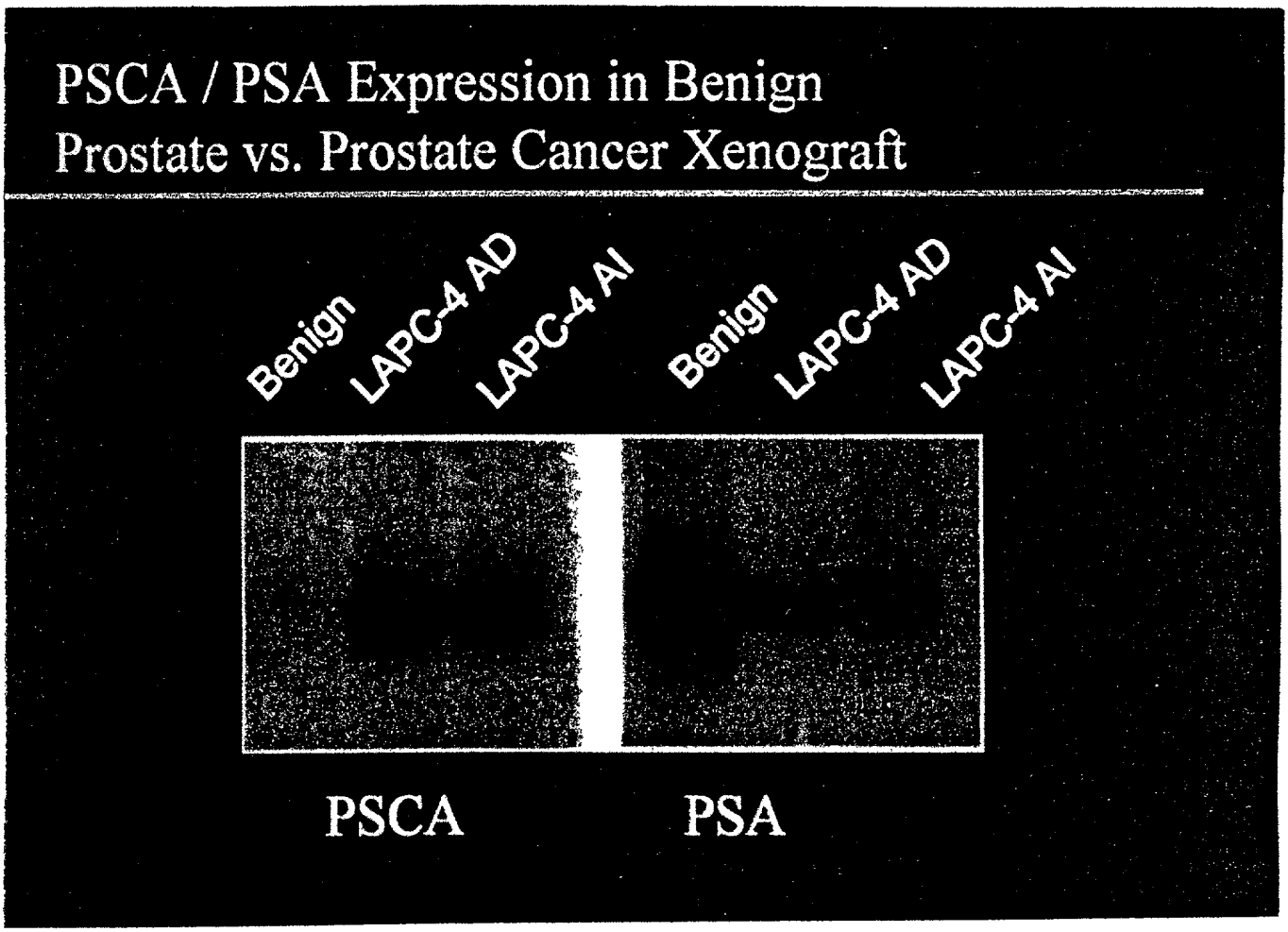

PSCA / PSA Expression in Benign Prostate vs. Prostate Cancer Xenograft
Benign
LAPC-4 AD
LAPC-4 AI
Benign
LAPC-4 AD
LAPC-4 AI
PSCA
PSA

FIGURE 9A

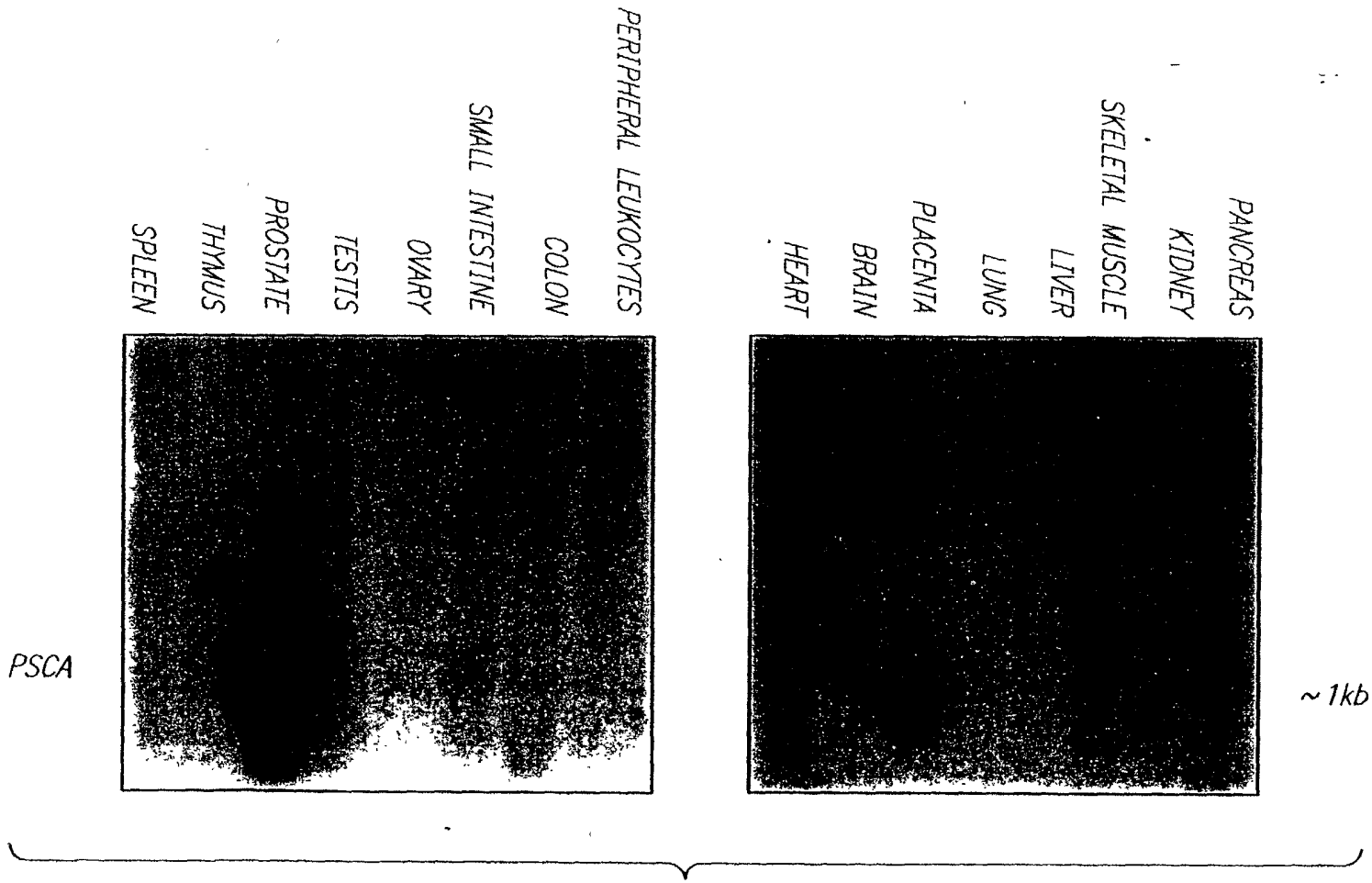


FIG. 9B

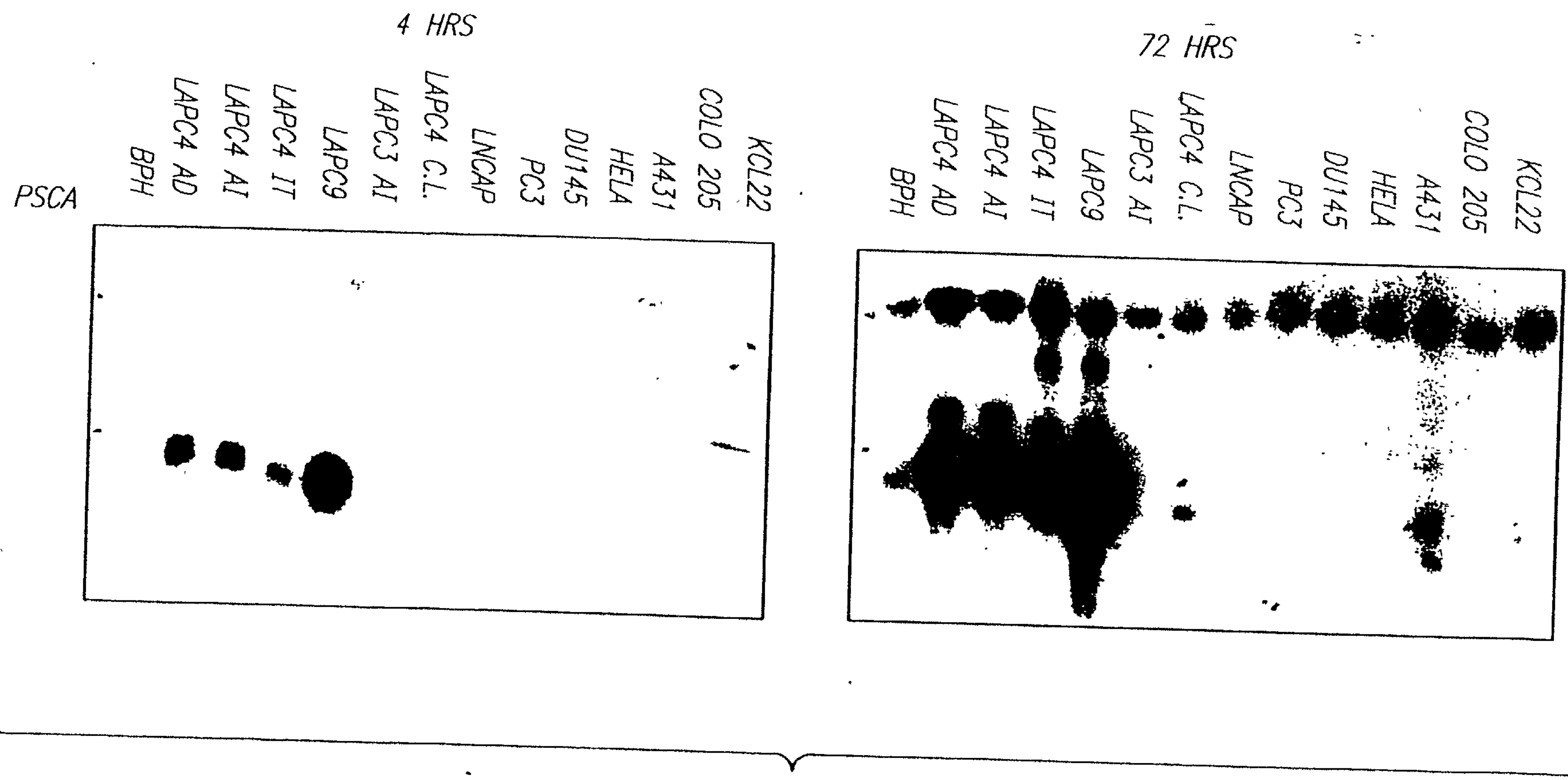

PSCA
4 HRS
BPH
LAPC4 AD
LAPC4 AI
LAPC4 IT
LAPC9
LAPC3 AI
LAPC4 C.L.
LNCAP
PC3
DU145
HELA
A431
COLO 205
KCL22
72 HRS
BPH
LAPC4 AD
LAPC4 AI
LAPC4 IT
LAPC9
LAPC3 AI
LAPC4 C.L.
LNCAP
PC3
DU145
HELA
A431
COLO 205
KCL22

FIG. 10-1

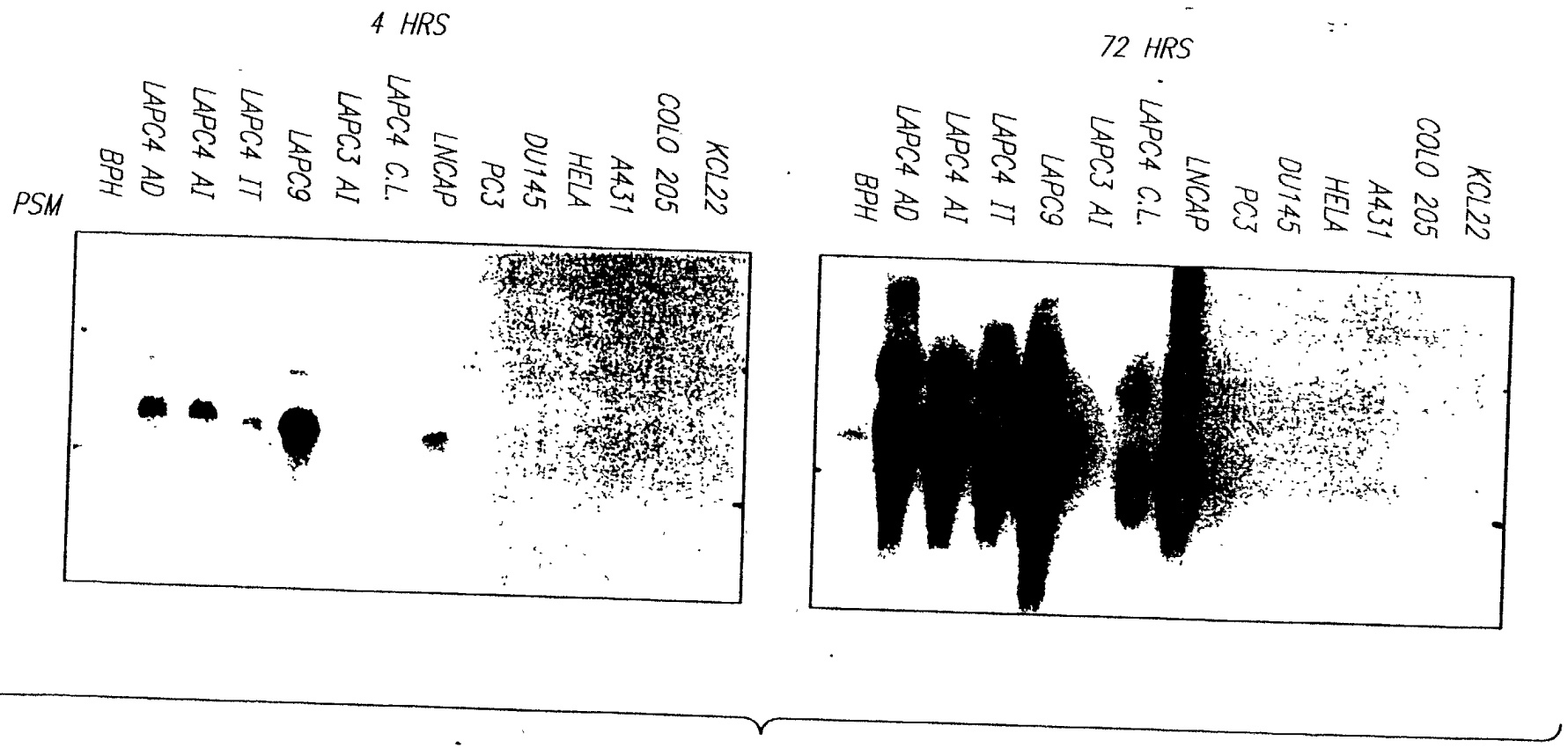


FIG. 10–2

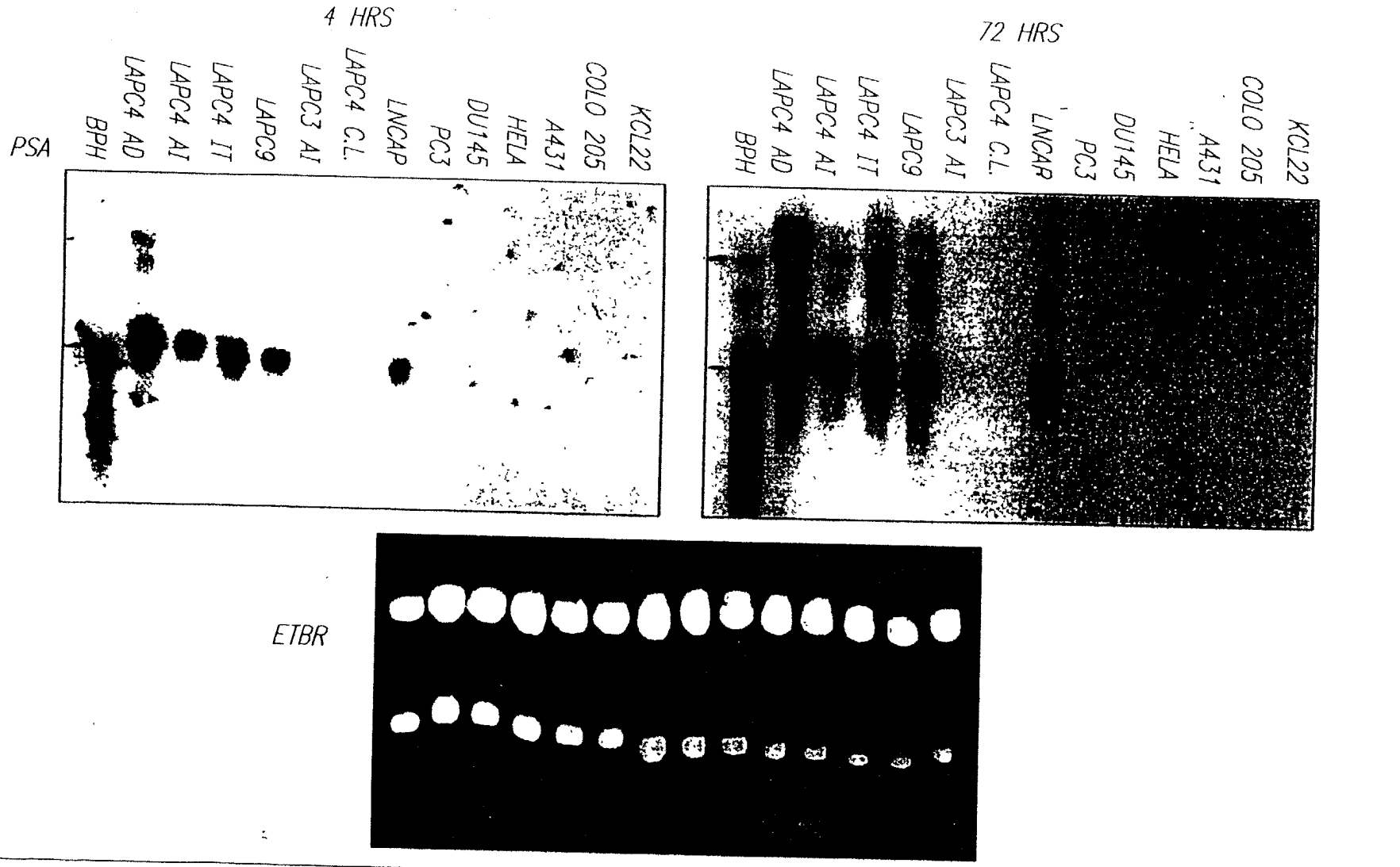


FIG. 10-3

FIG. 11A

FIG. 11B

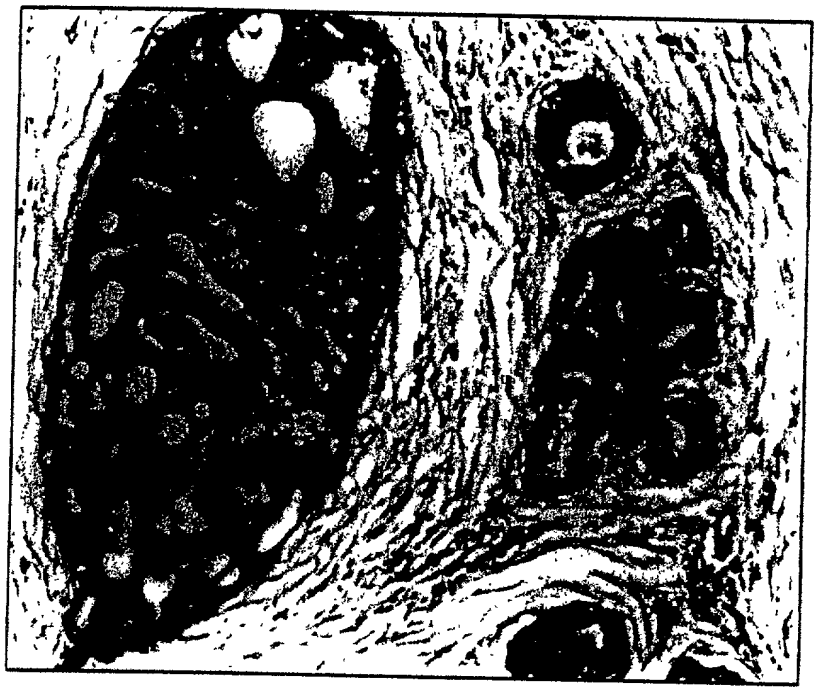
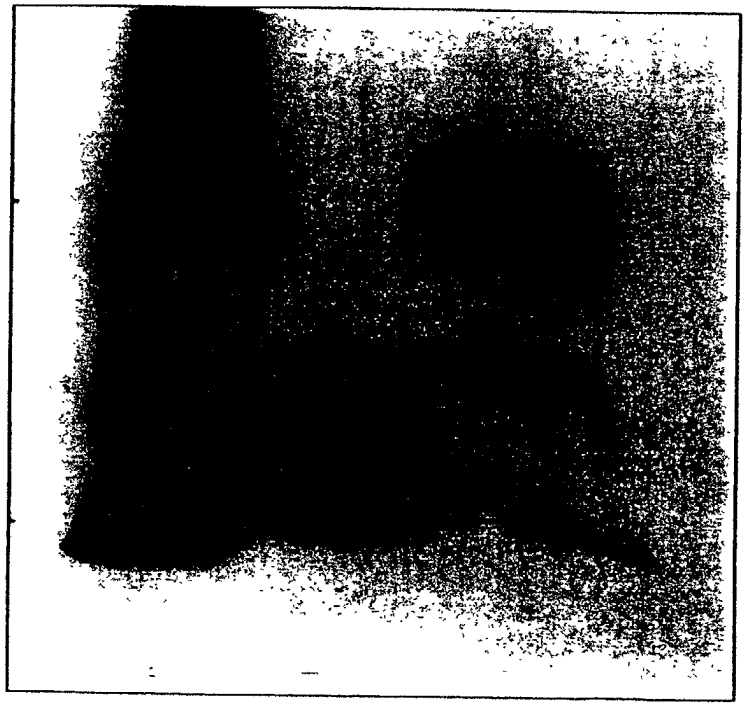


FIG. 11C

FIG. 12A



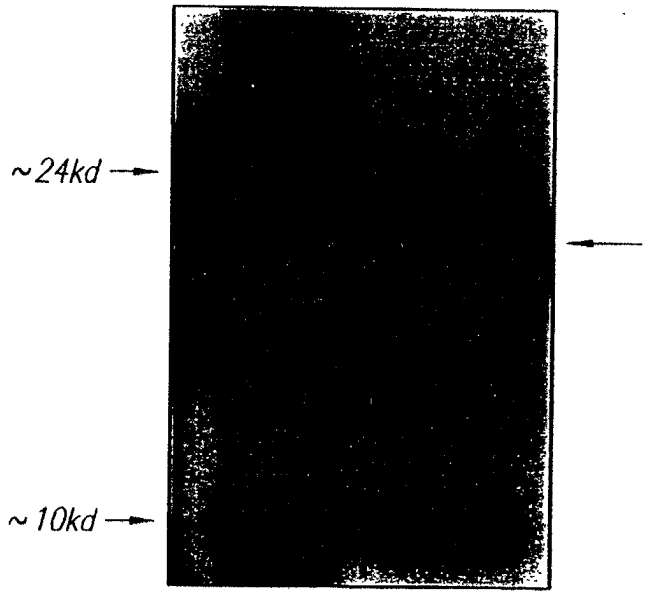


FIG. 12B

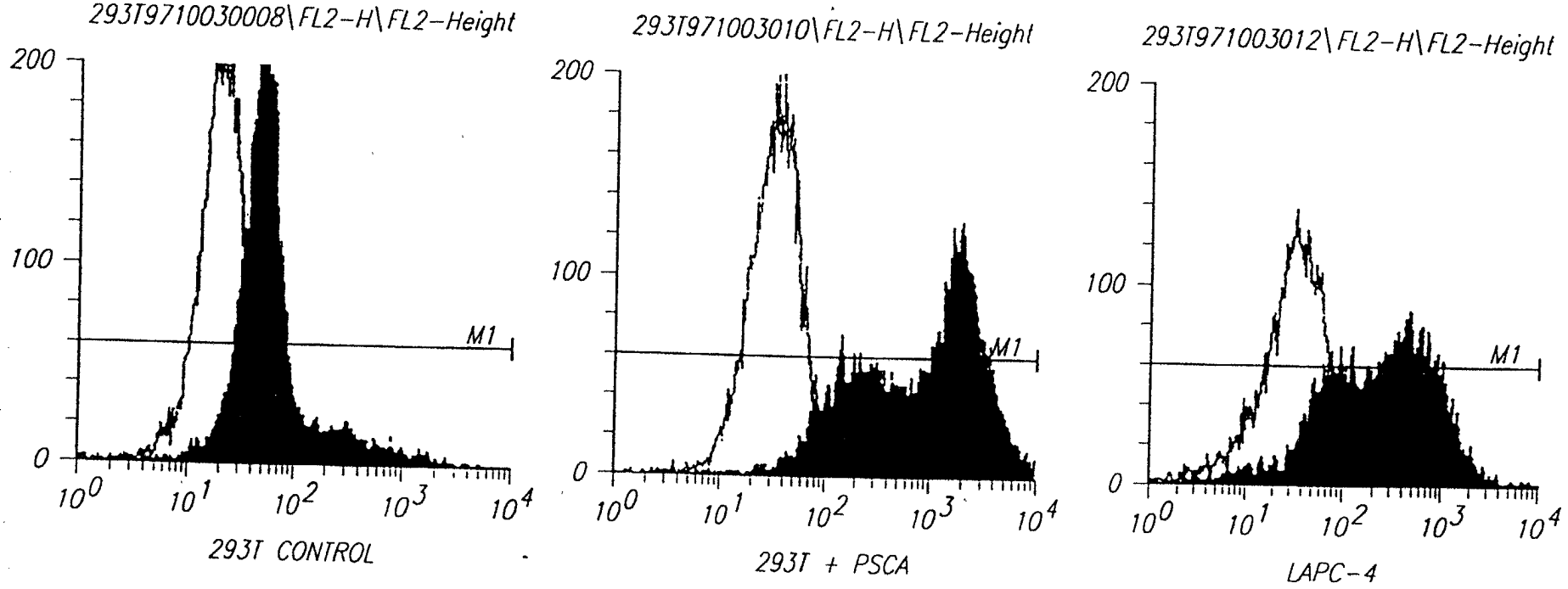


FIGURE 12C

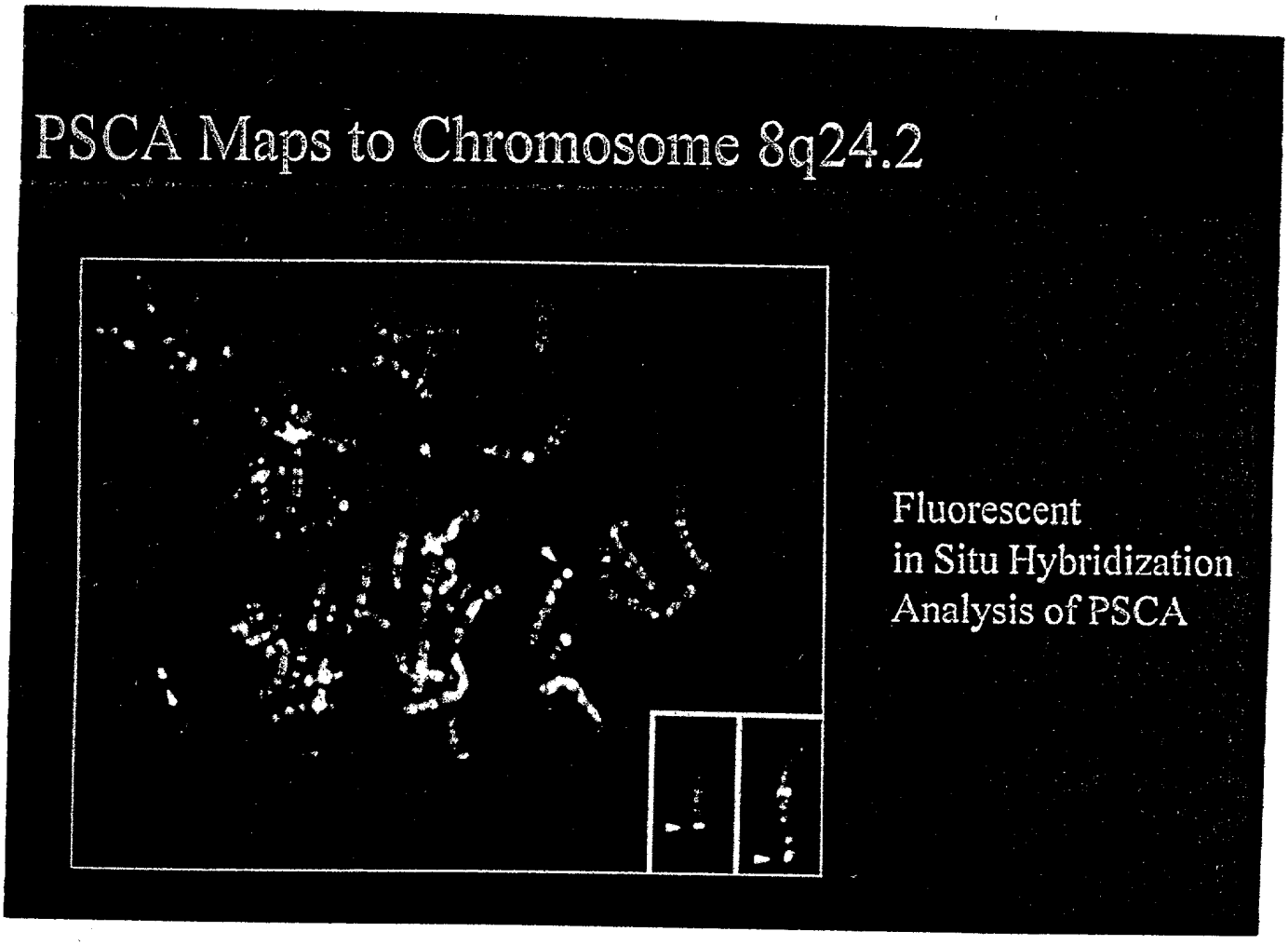

PSCA Maps to Chromosome 8q24.2
Fluorescent
in Situ Hybridization
Analysis of PSCA

FIGURE 13

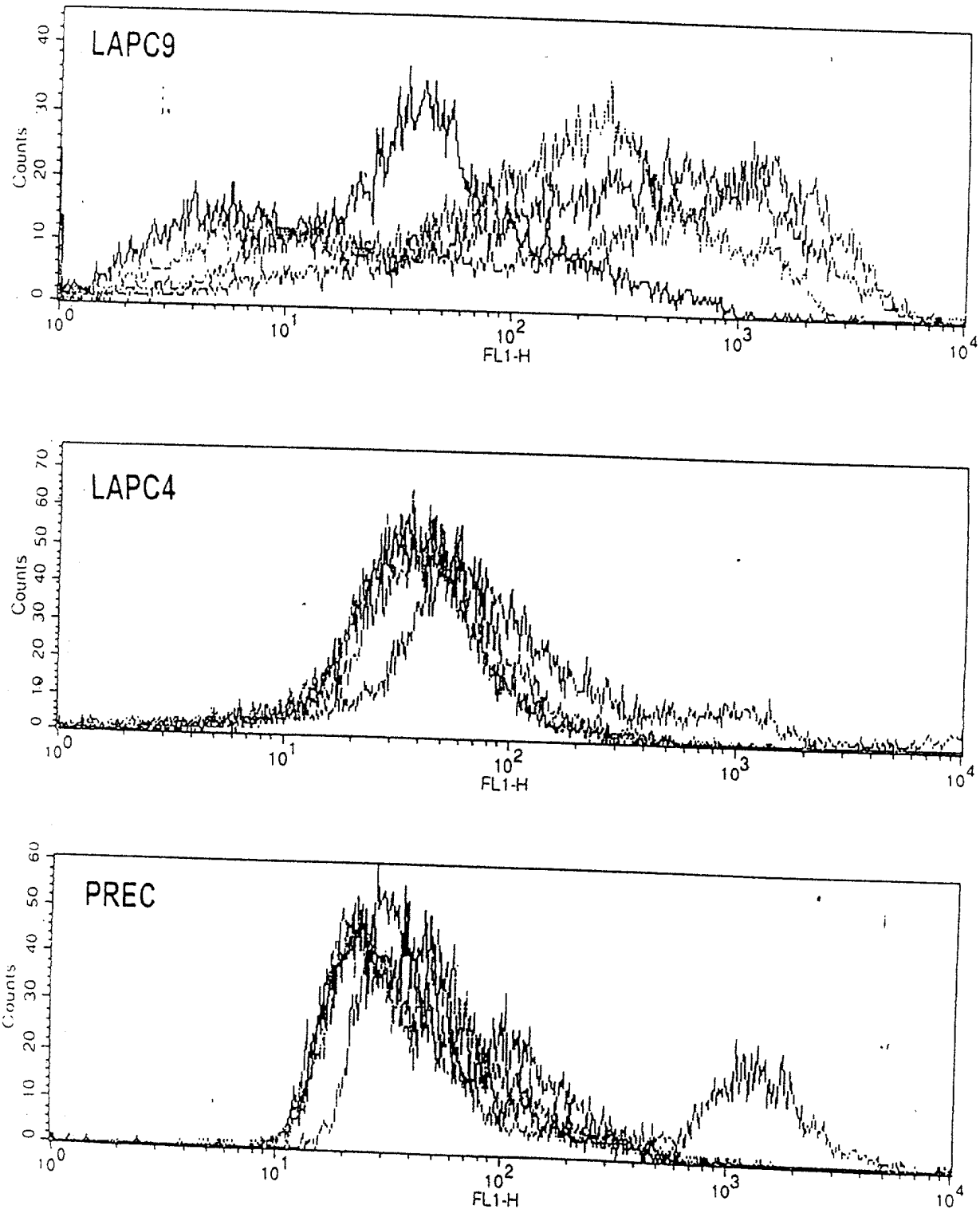


FIGURE 14

A

Epitope map

mAb	Isotype		FL (18-98)	N (2-50)	M (46-109)	C (85-123)
1G8	IgG1	k	**2.039**	0.007	**0.628**	0.000
2H9	IgG1	k	**1.318**	**0.863**	0.032	0.021
3C5	IgG2a	k	**2.893**	**1.965**	0.016	0.005
3E6	IgG3	k	**0.328**	0.024	**0.069**	**0.370**
4A10	IgG2a	k	**2.039**	**1.315**	0.000	0.014
2A2	IgG2a	k	**1.366**	**0.733**	0.010	0.003
3G3	IgG2a	k	**2.805**	**1.731**	0.004	0.000

B

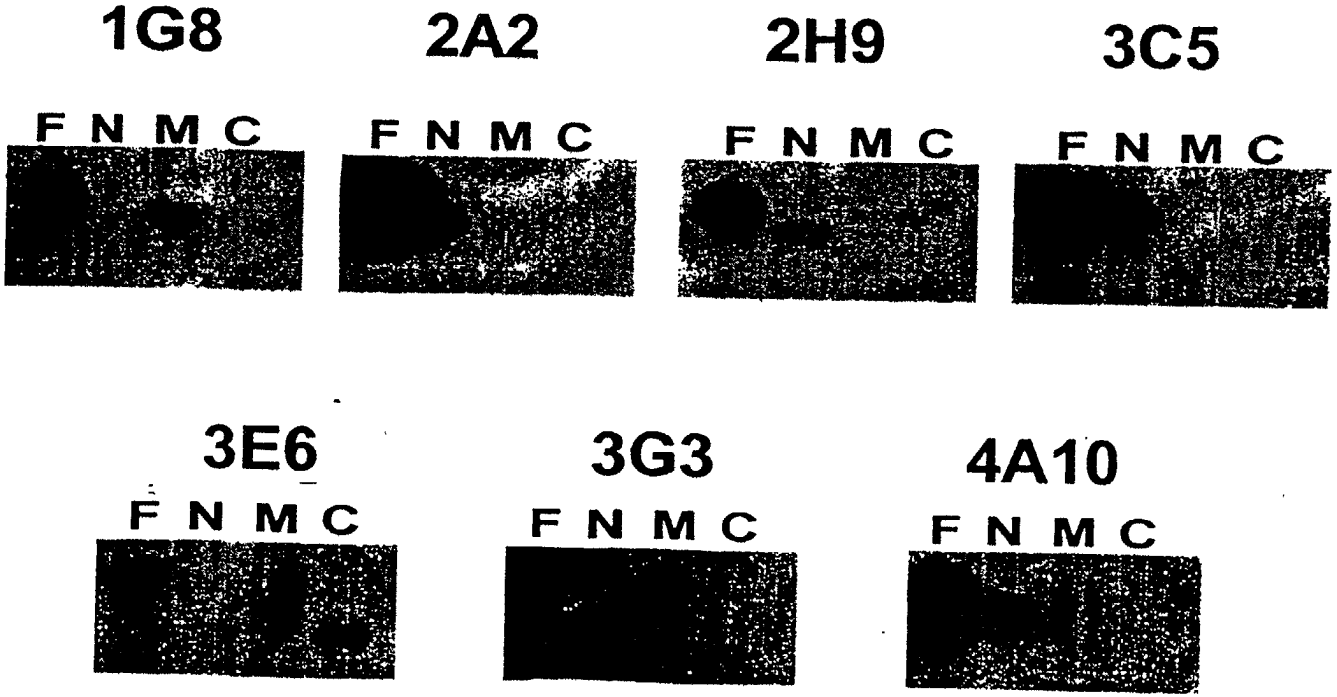
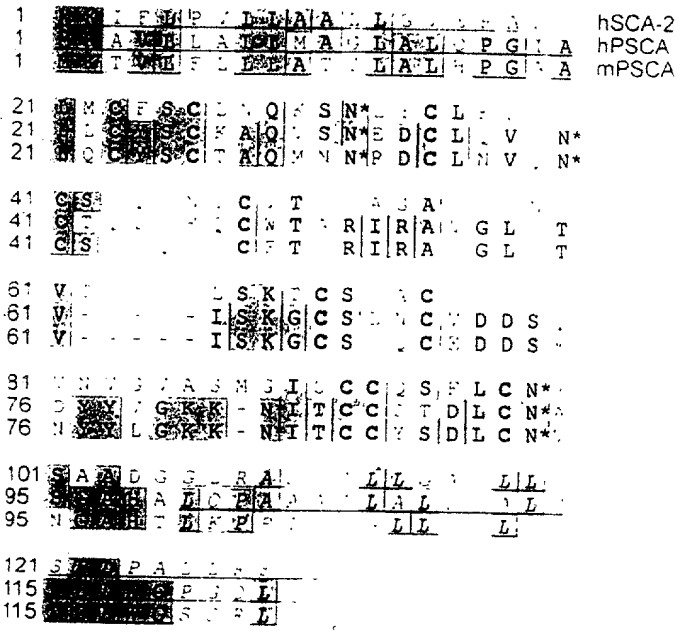


FIGURE 15

Prostate Stem Cell Antigen (PSCA) is a GPI-anchored Protein



(Reiter, R.E., et al., 1997. *PNAS*)

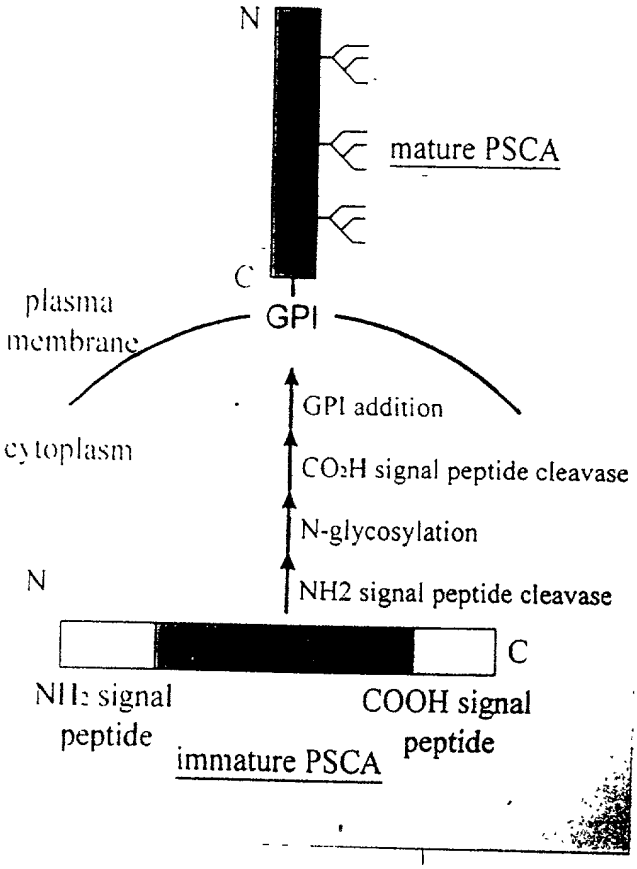


FIGURE 16

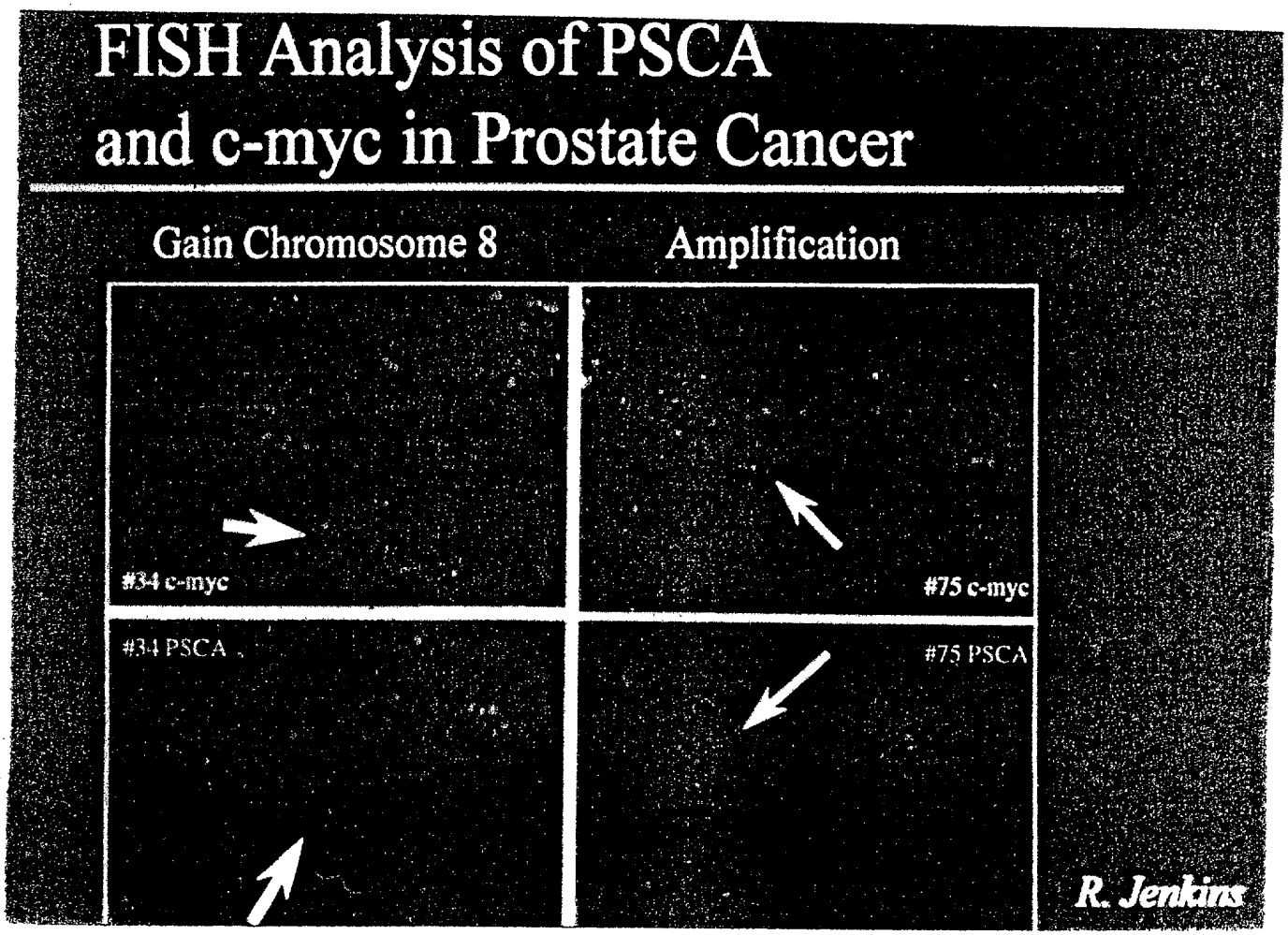

FISH Analysis of PSCA
and c-myc in Prostate Cancer
Gain Chromosome 8
Amplification
#34 c-myc
#75 c-myc
#34 PSCA
#75 PSCA
R. Jenkins

FIGURE 17

FIGURE 18

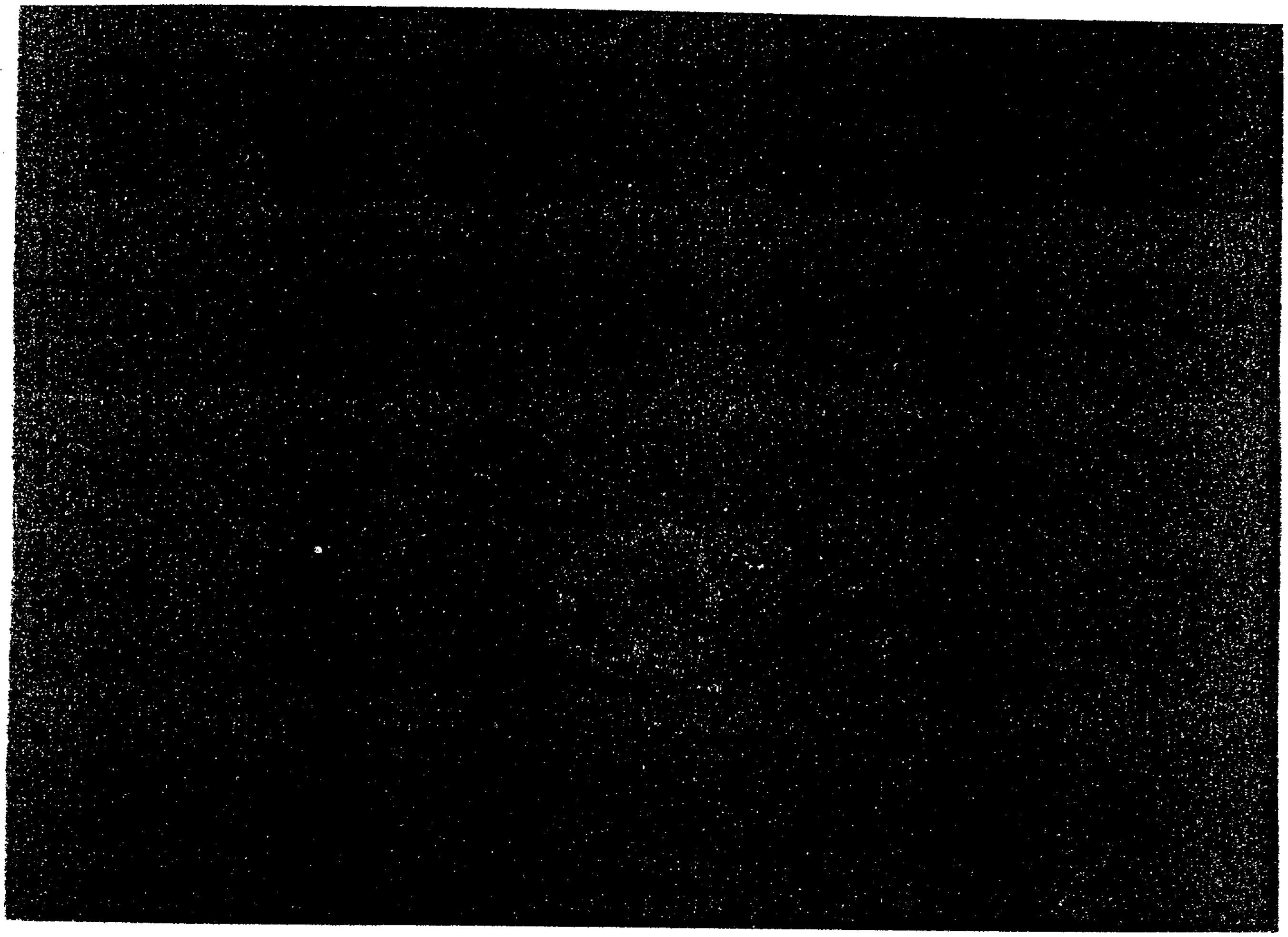


FIGURE 19

FIGURE 20

PSCA Immunostaining of Primary Tumors

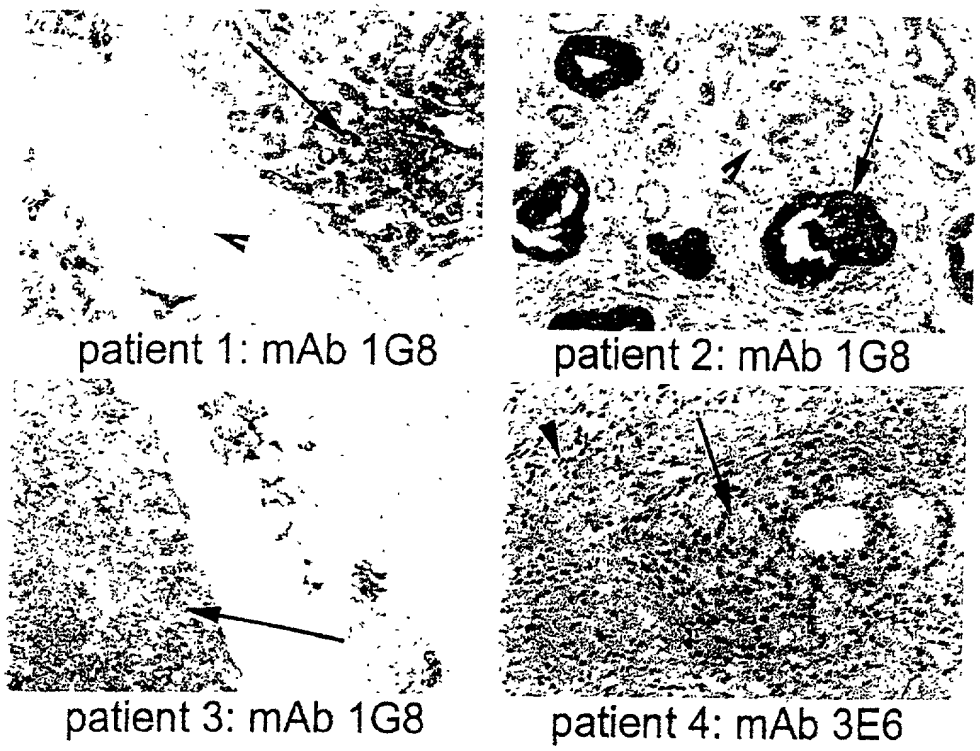


FIGURE 21

FIGURE 22

FIGURE 23

FIGURE 24

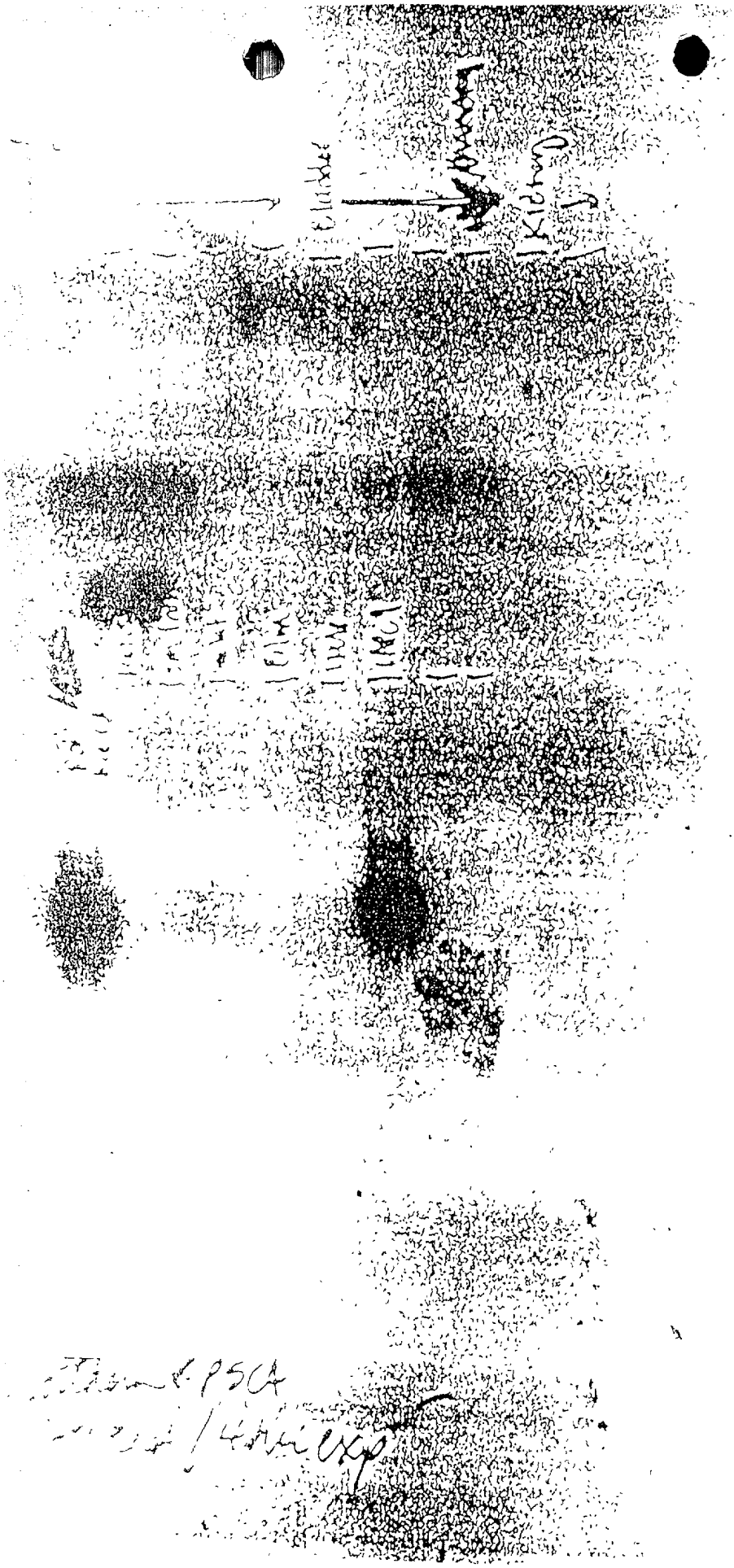


FIGURE 25

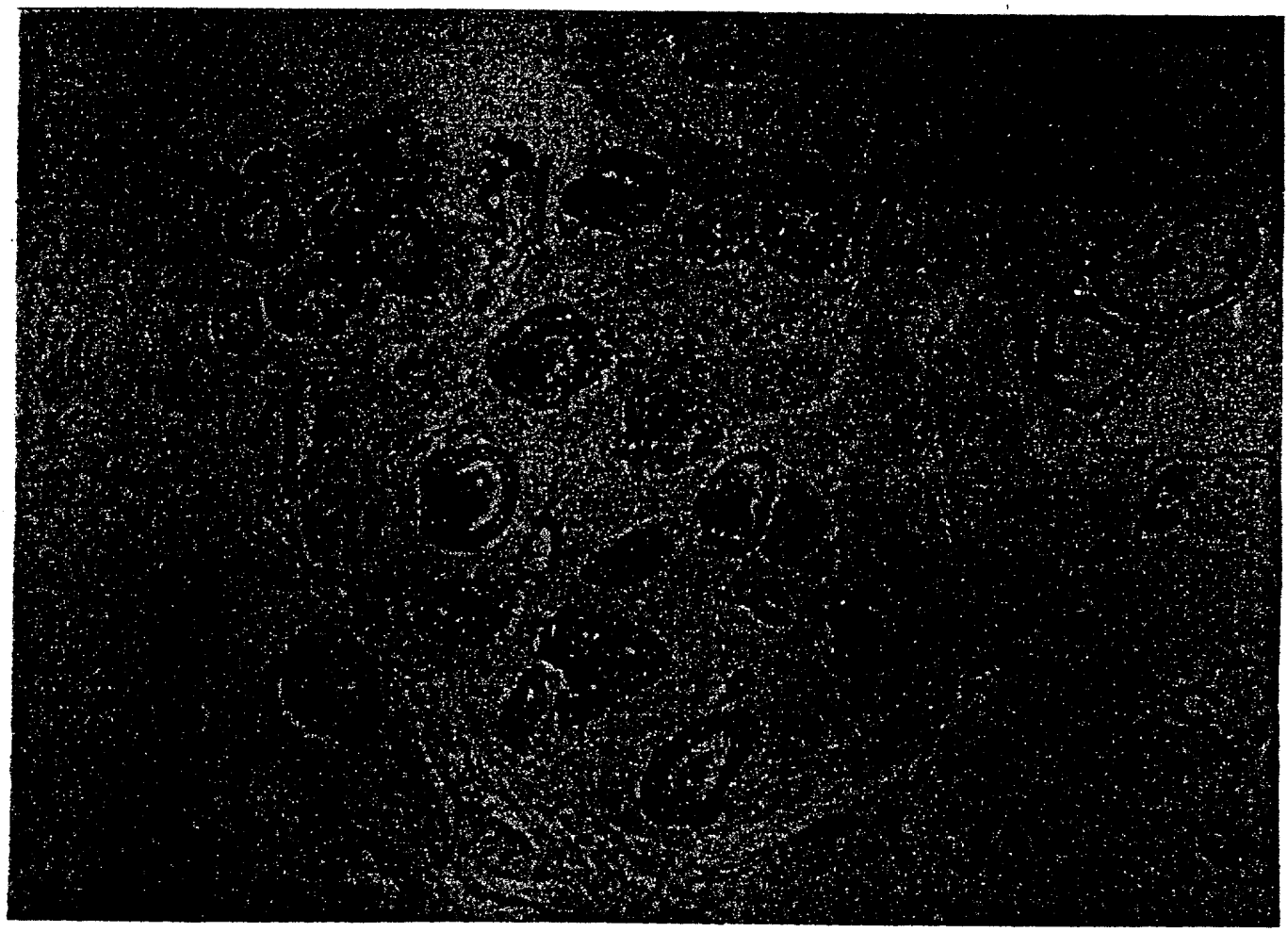


FIGURE 26

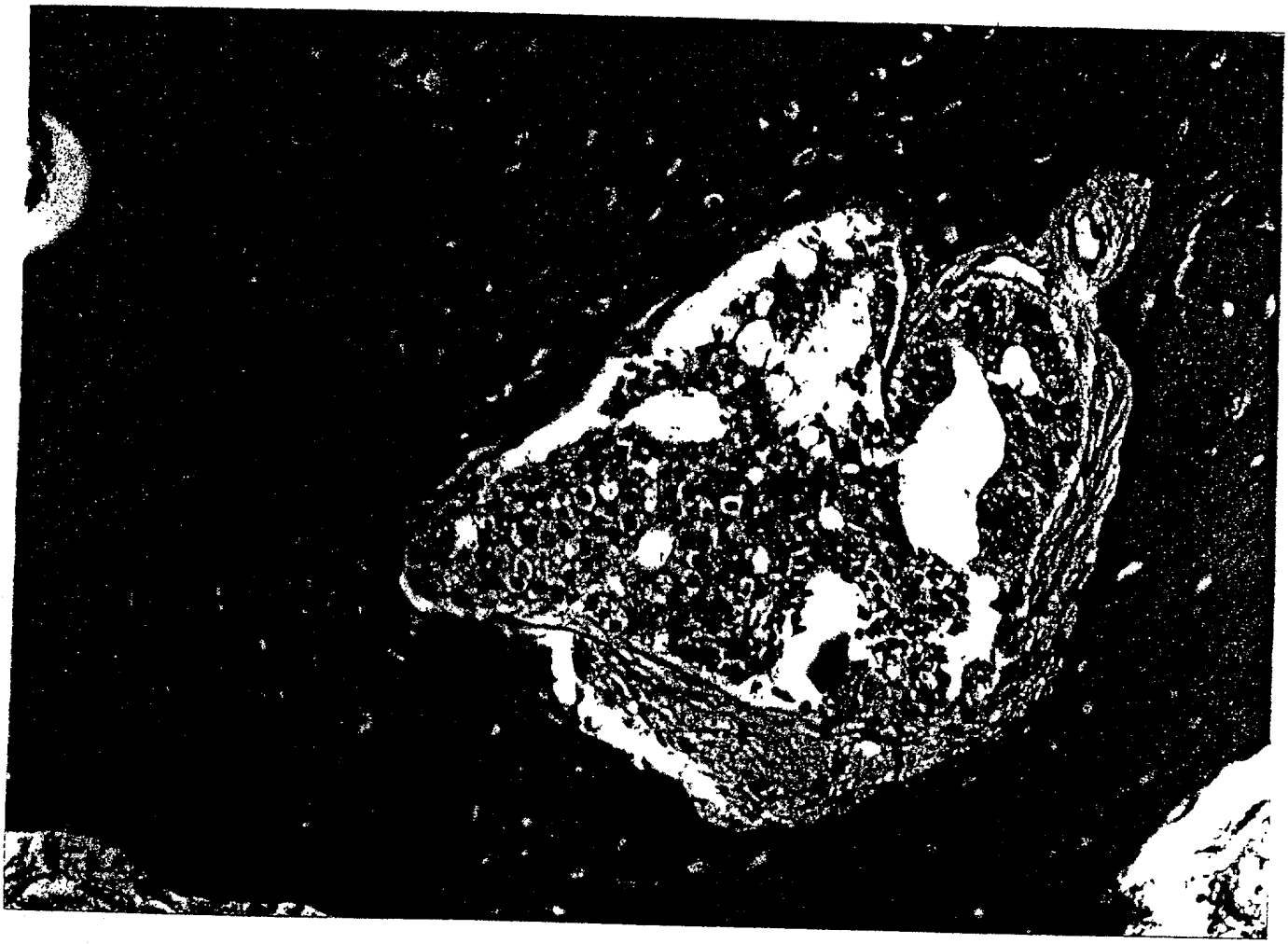
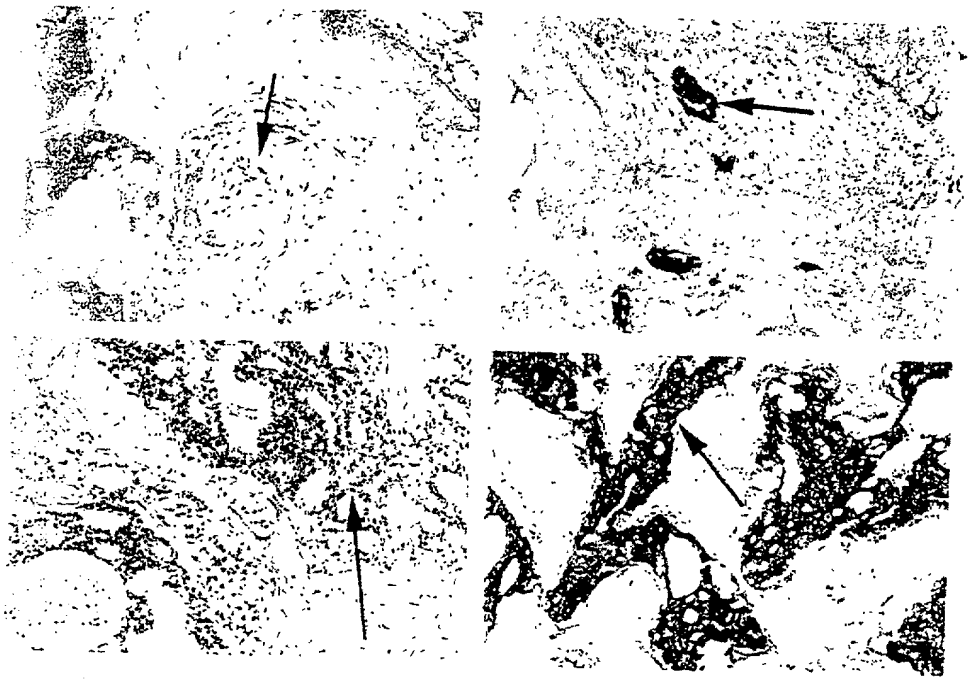


FIGURE 27

PSCA Immunostaining of Bony Metastases



Patient 5: H and E and mAb 1G8

Patient 4: H and E and mAb 3E6

FIGURE 28

FIGURE 29

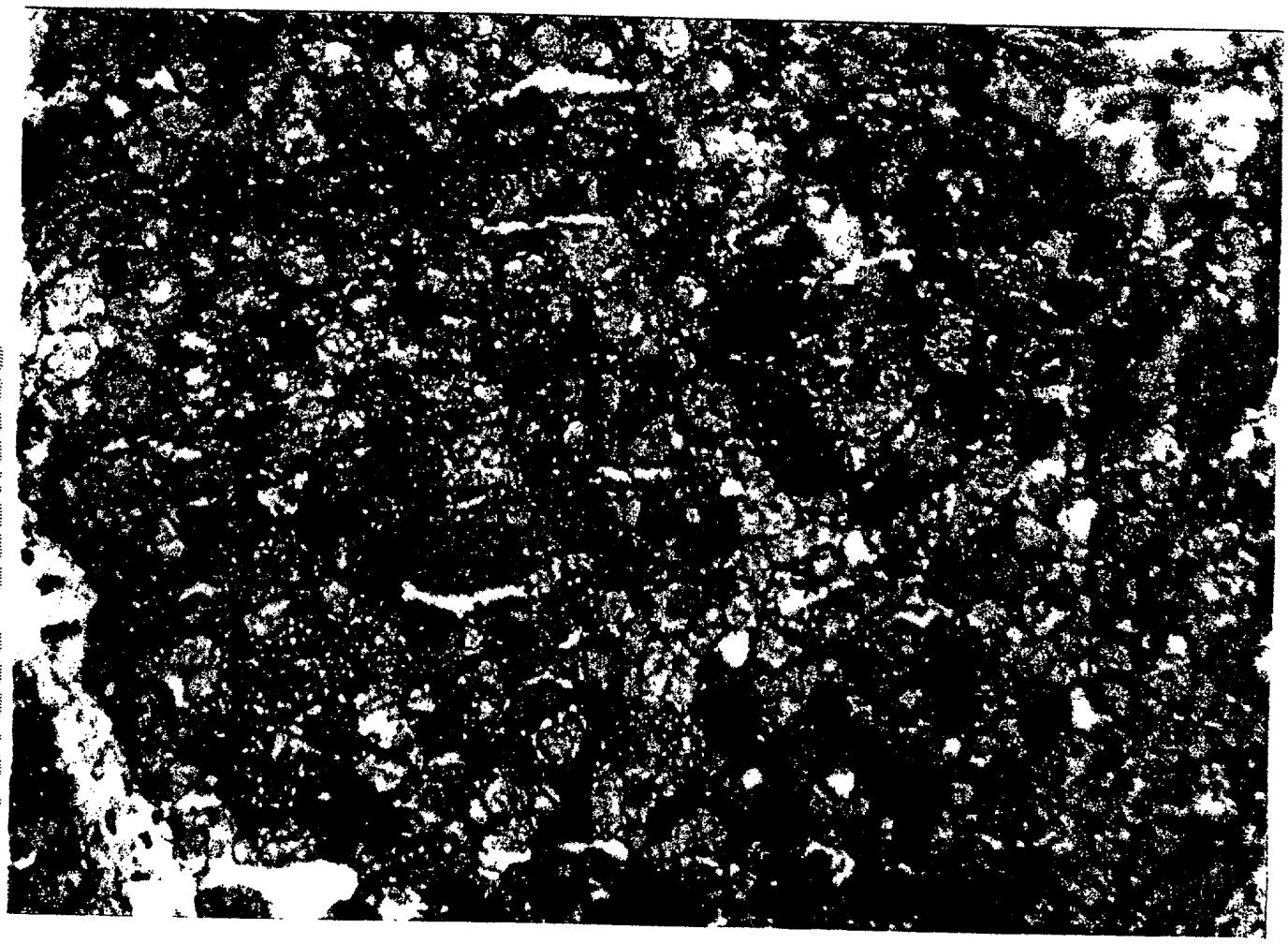


FIGURE 30

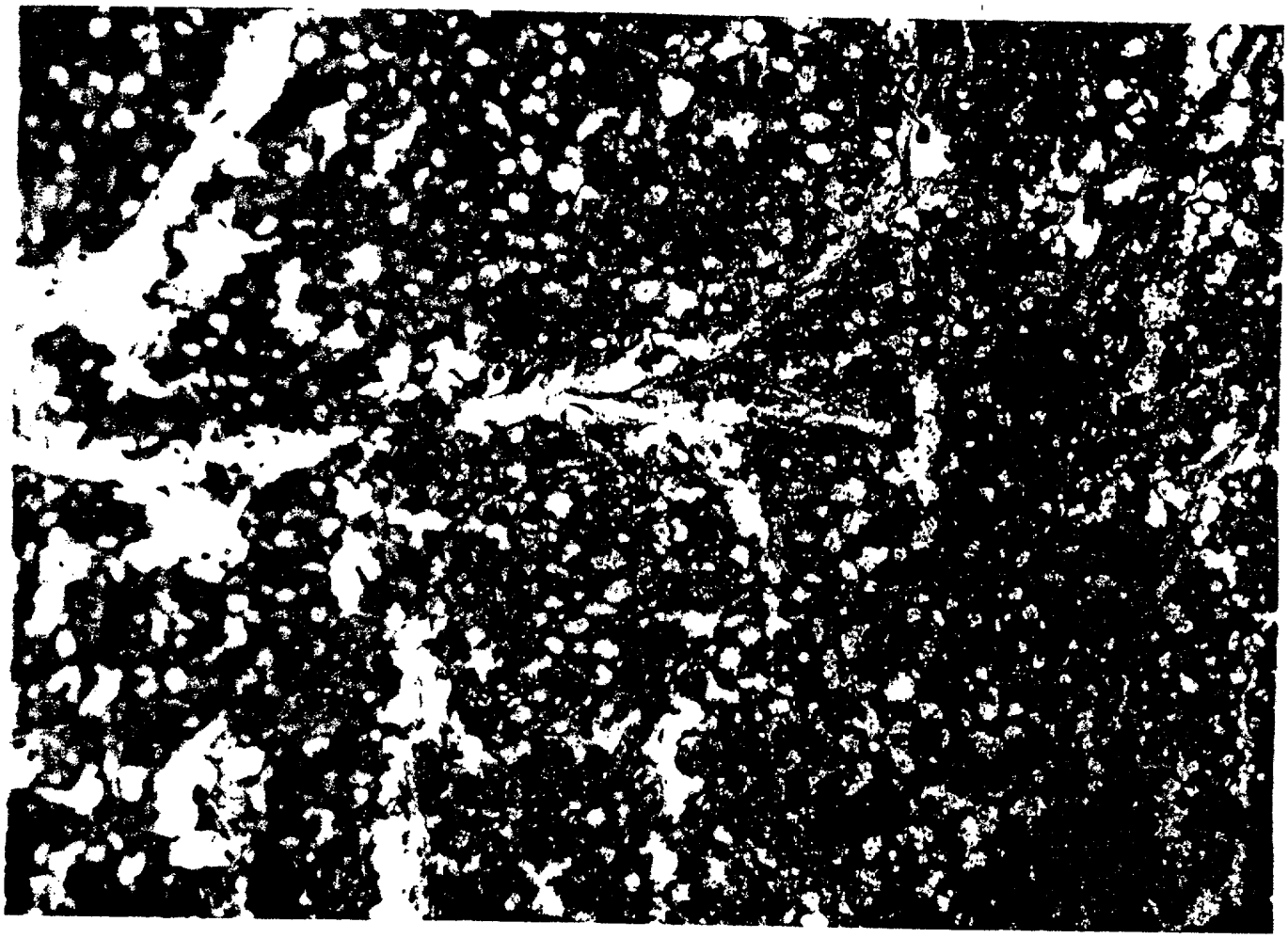


FIGURE 31

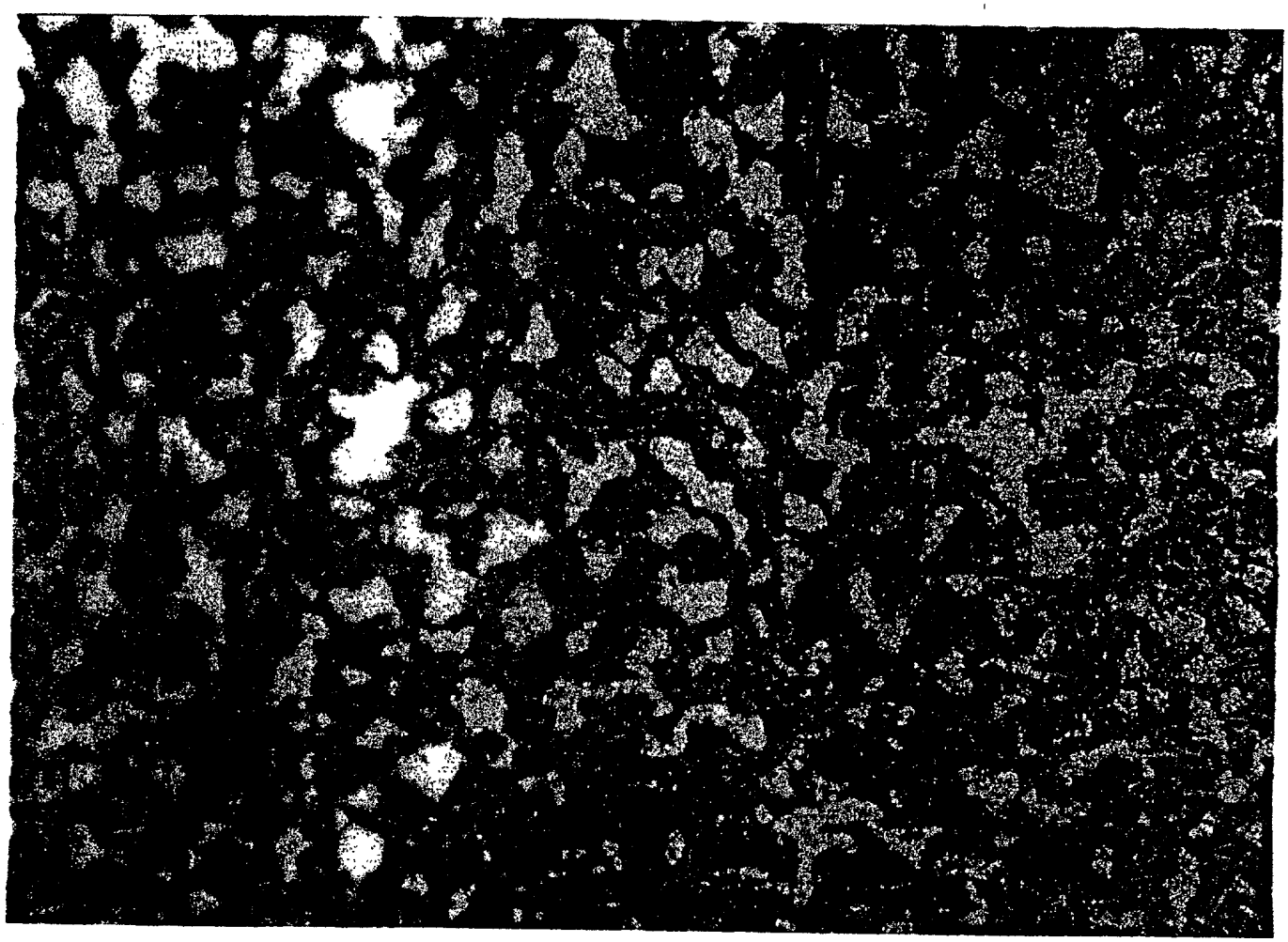


FIGURE 32

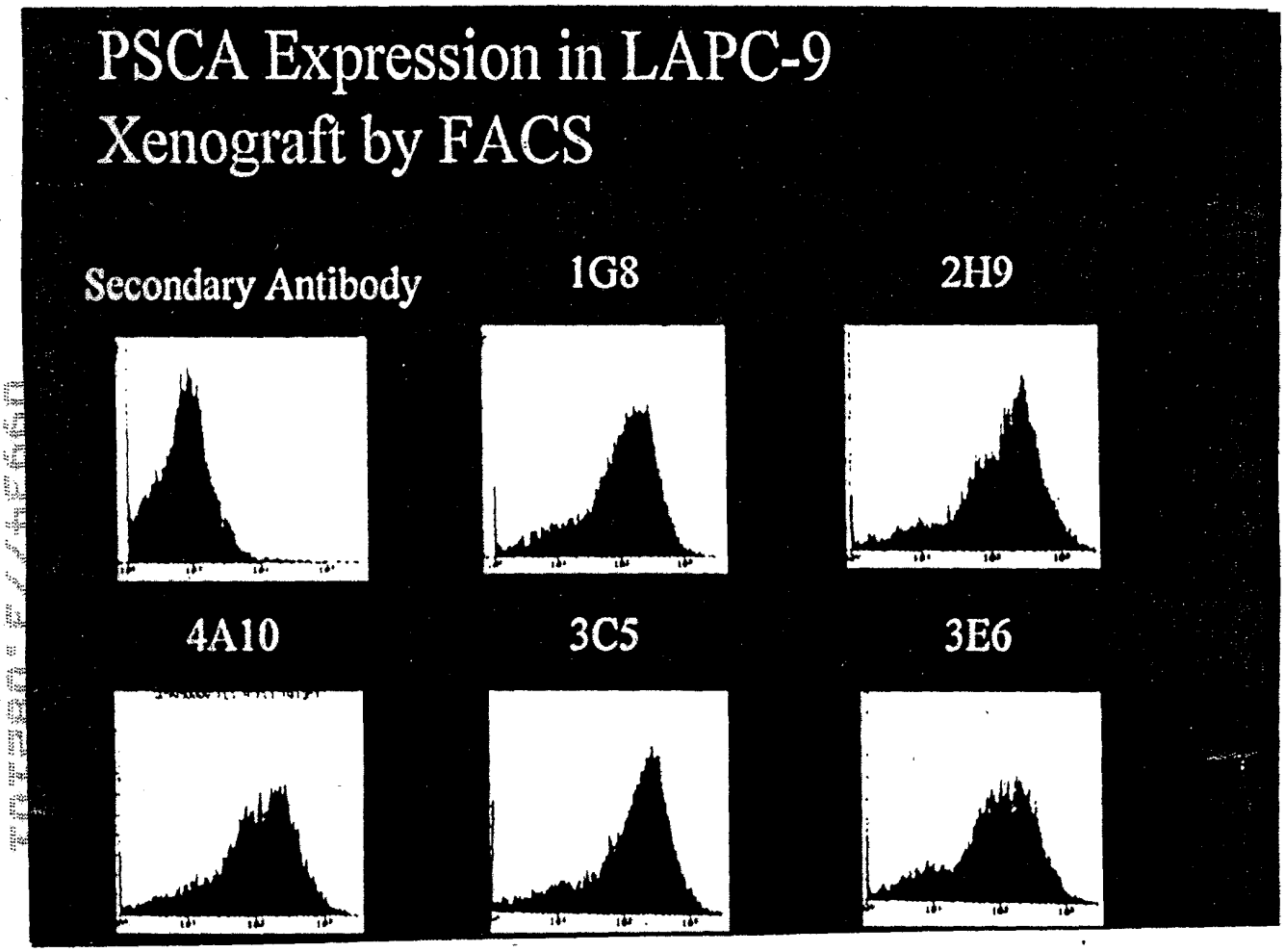

PSCA Expression in LAPC-9
Xenograft by FACS
Secondary Antibody
1G8
2H9
4A10
3C5
3E6

FIGURE 33

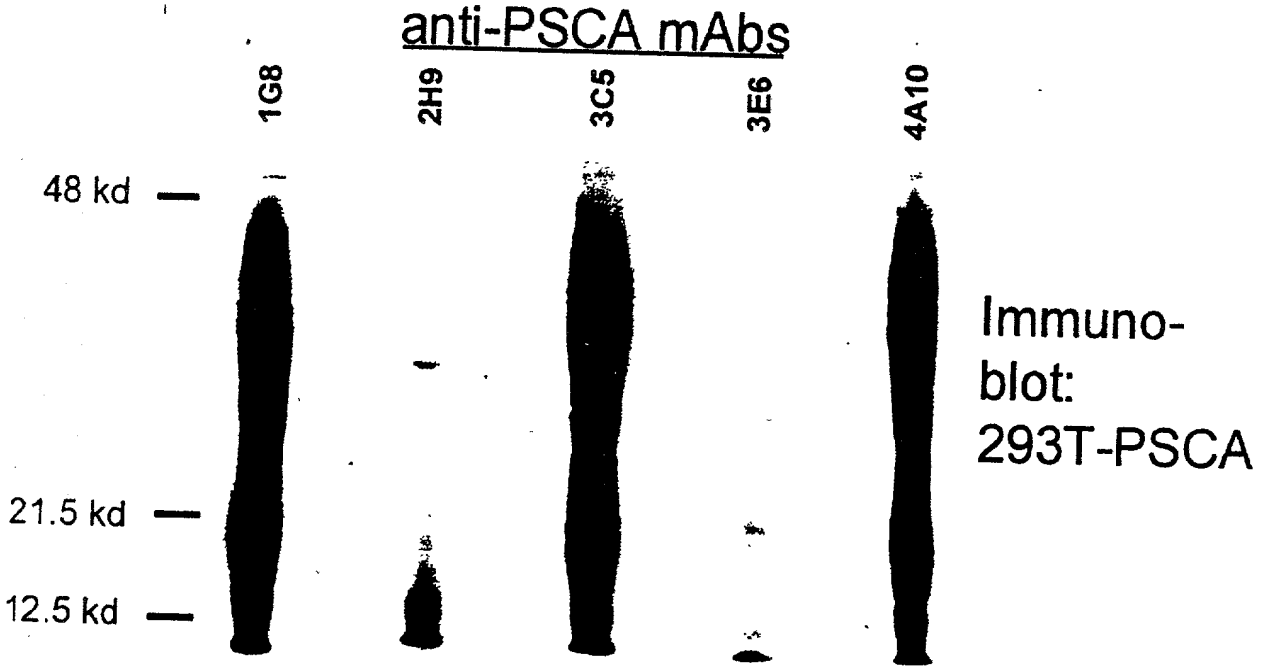

anti-PSCA mAbs
1G8
2H9
3C5
3E6
4A10
48 kd
21.5 kd
12.5 kd
Immuno-
blot:
293T-PSCA

FIGURE 34

Immunofluorescent Staining of LNCaP-PSCA Cells

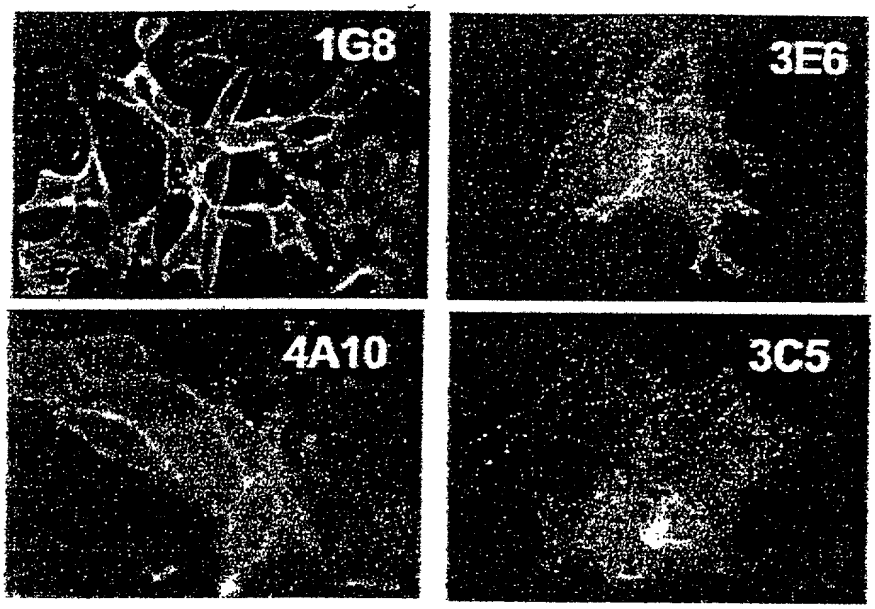


FIGURE 35

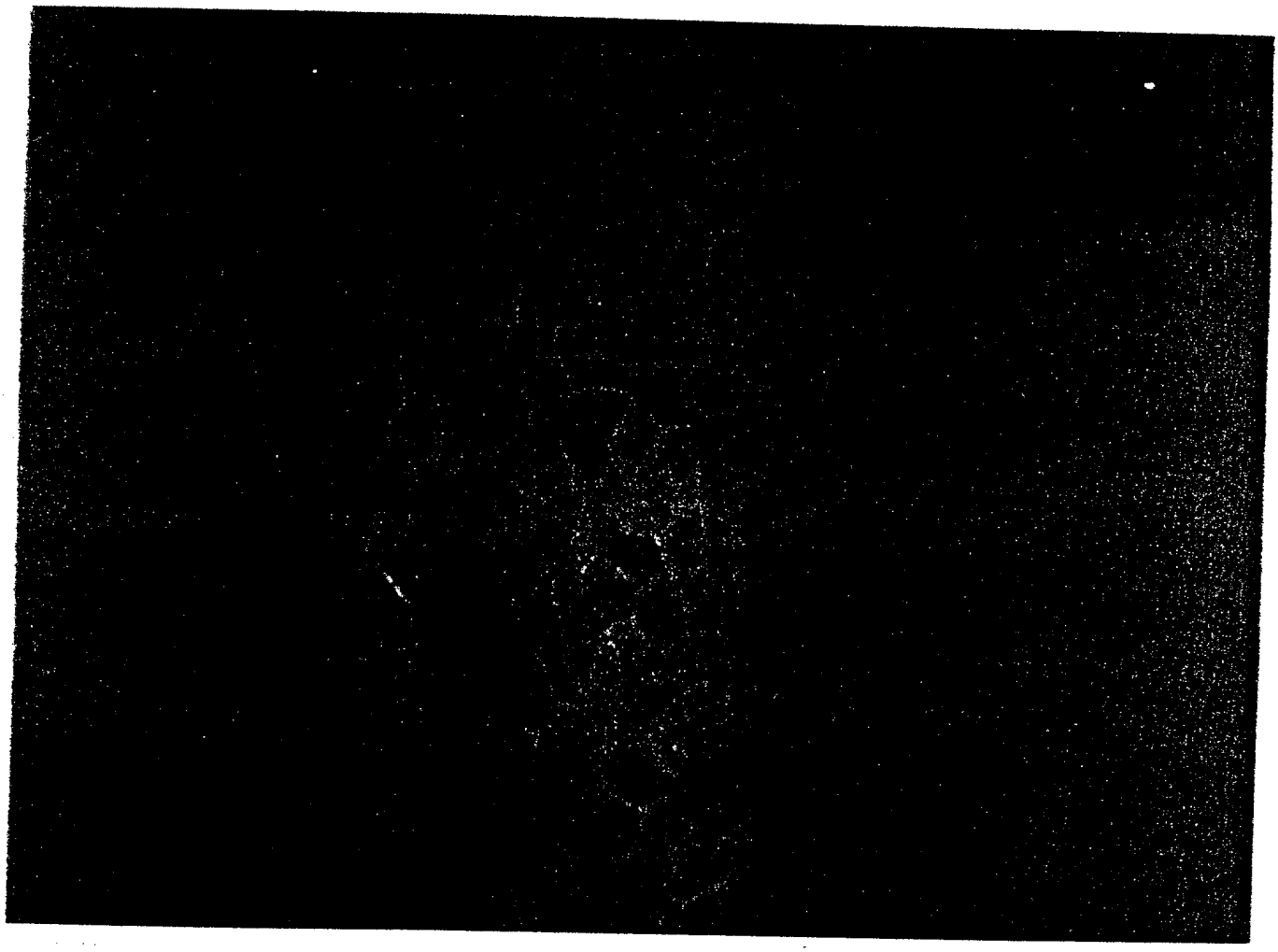


FIGURE 36

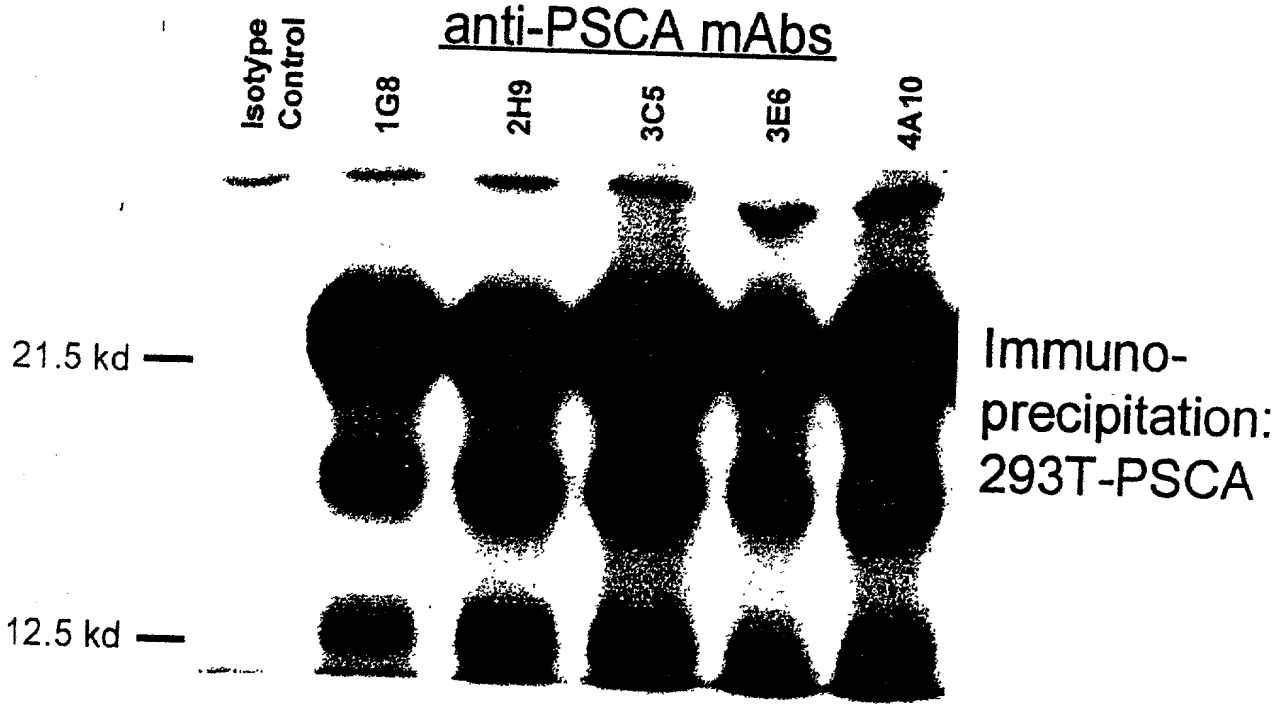
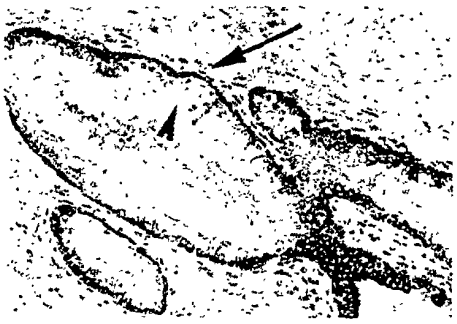

anti-PSCA mAbs
Isotype Control
1G8
2H9
3C5
3E6
4A10
21.5 kd
12.5 kd
Immuno-precipitation:
293T-PSCA

FIGURE 37

Immunohistochemical Staining of Normal Prostate

Normal: Isotype Control

Normal: PSCA mAb 3E6



Normal: PSCA mAb 1G8

Atrophy: PSCA mAb 2H9

FIGURE 38

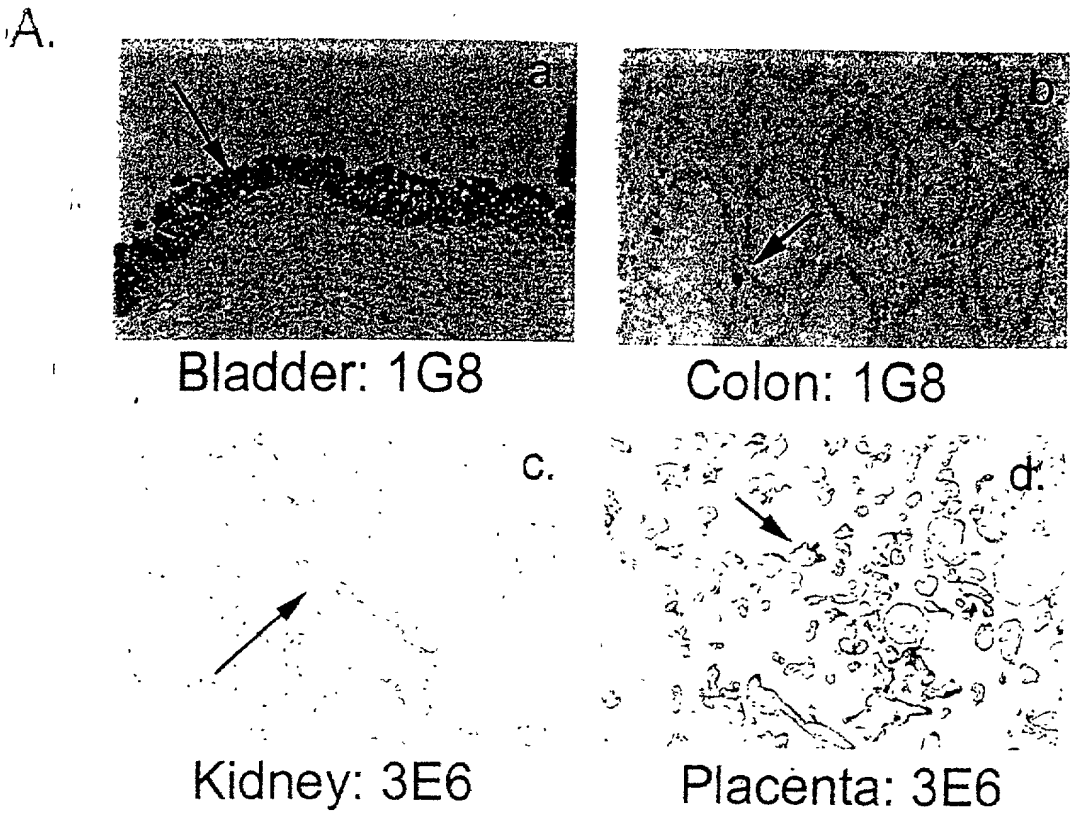
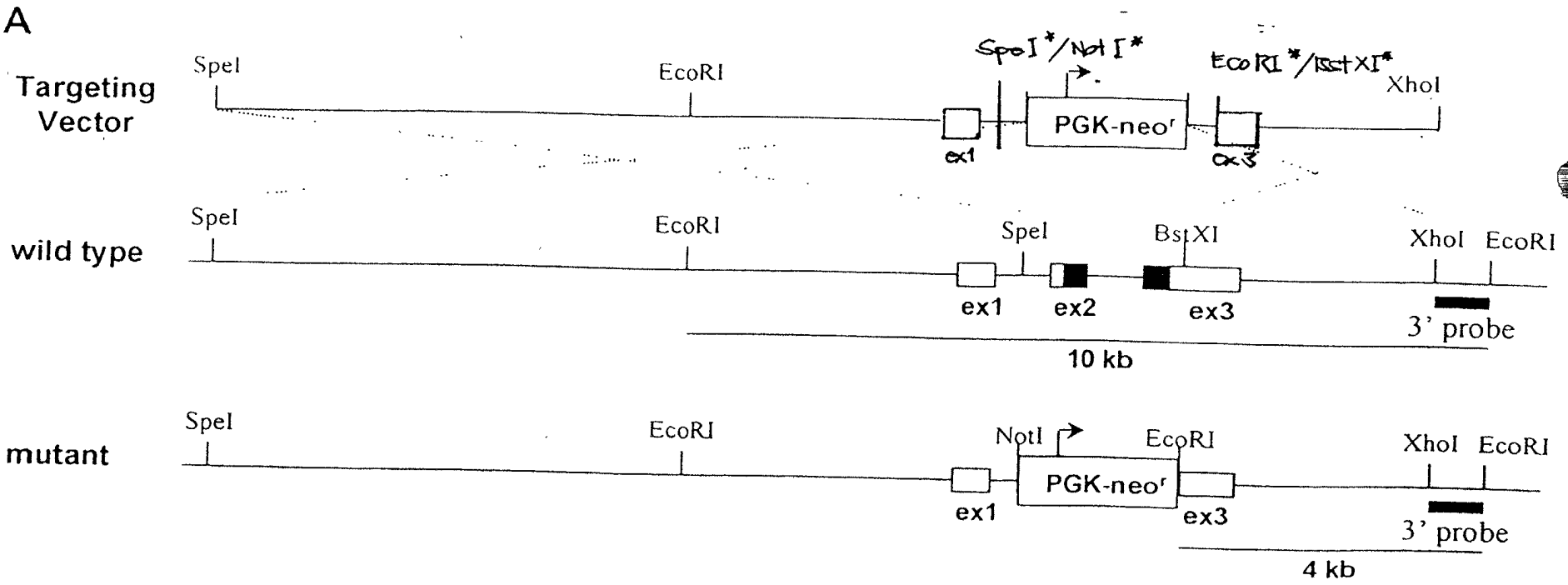

A.
a
b
Bladder: 1G8
Colon: 1G8
c.
d.
Kidney: 3E6
Placenta: 3E6
B.
Prostate
Prostate
Prostate
Kidney
Kidney
Kidney
Bladder
Bladder
Bladder
LAPC 9
PSCA
Actin

FIGURE 39

Targeting of Mouse PSCA Gene



* ex1, 2, and 3 are the exons of PSCA gene.
* Black boxes of ex2 and ex3 encode PSCA mature protein sequences.
* ES genomic DNA's were digested with EcoRI, followed by Southern hybridization using 3' probe.

B. Genomic Southern Analysis of ES Cells

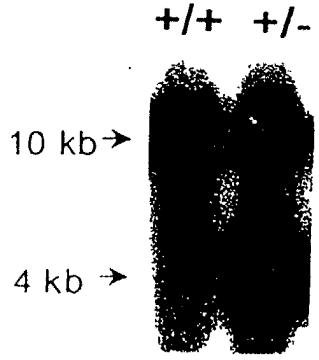
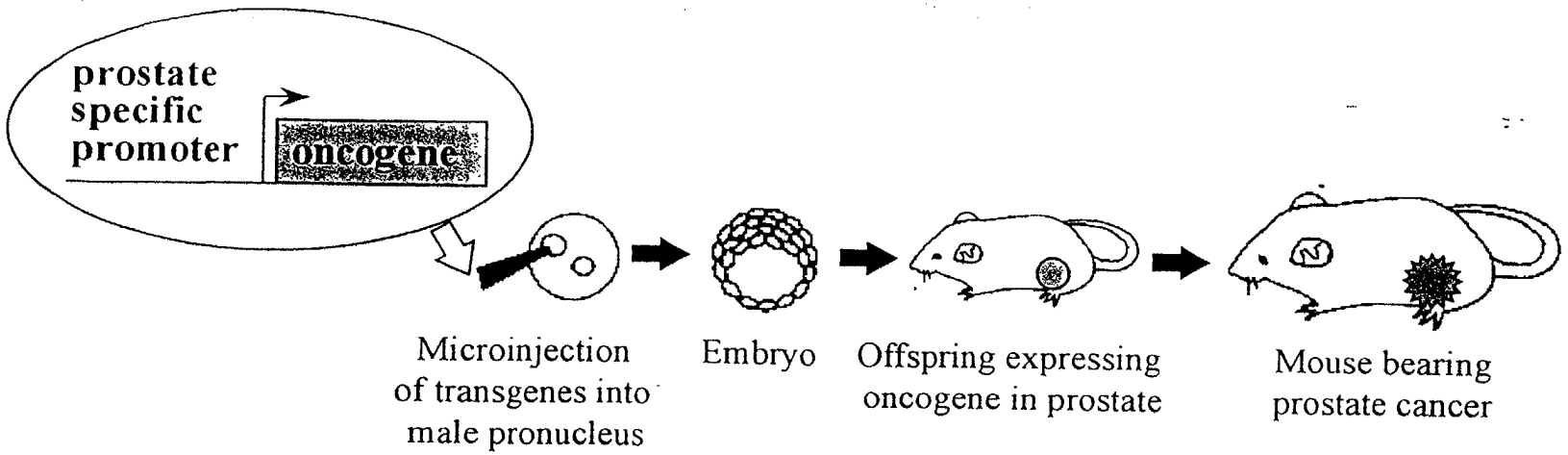


FIGURE 40

Transgenic Mouse Models of Prostate Cancer



Transgene	Target tissues	Characteristics
C3(1) (-3 kb)/ SV40 large+small T *Maroulakou et al.* *1994 PNAS*	prostate (secretory cells) urethral, mammary and sweat gland	Low-grade PIN 8-12 wks High-grade PIN 8-12 wks Invasive carcicinoma 28 wks No metastases
Probasin (-426 bp)/ SV40 large+small T *Greenberg et al.* *1995 PNAS*	prostate (secretory cells)	Low-grade PIN 5-8 wks High-grade PIN 8-12 wks Invasive carcicinoma 12 wks Metastases in lymph node, lung, liver and bone
Cryptdin2 (-6.5 kb)/ SV40 large+small T *Garabedian et al.* *1998 PNAS*	prostate (neuroendocrine cells) small intestine	Low-grade PIN 8-12 wks High-grade PIN 8-12 wks Invasive carcicinoma 16 wks Metastases in lymph node, lung, liver and bone

FIGURE 41

Reporter Gene Constructs for Transfection Assay

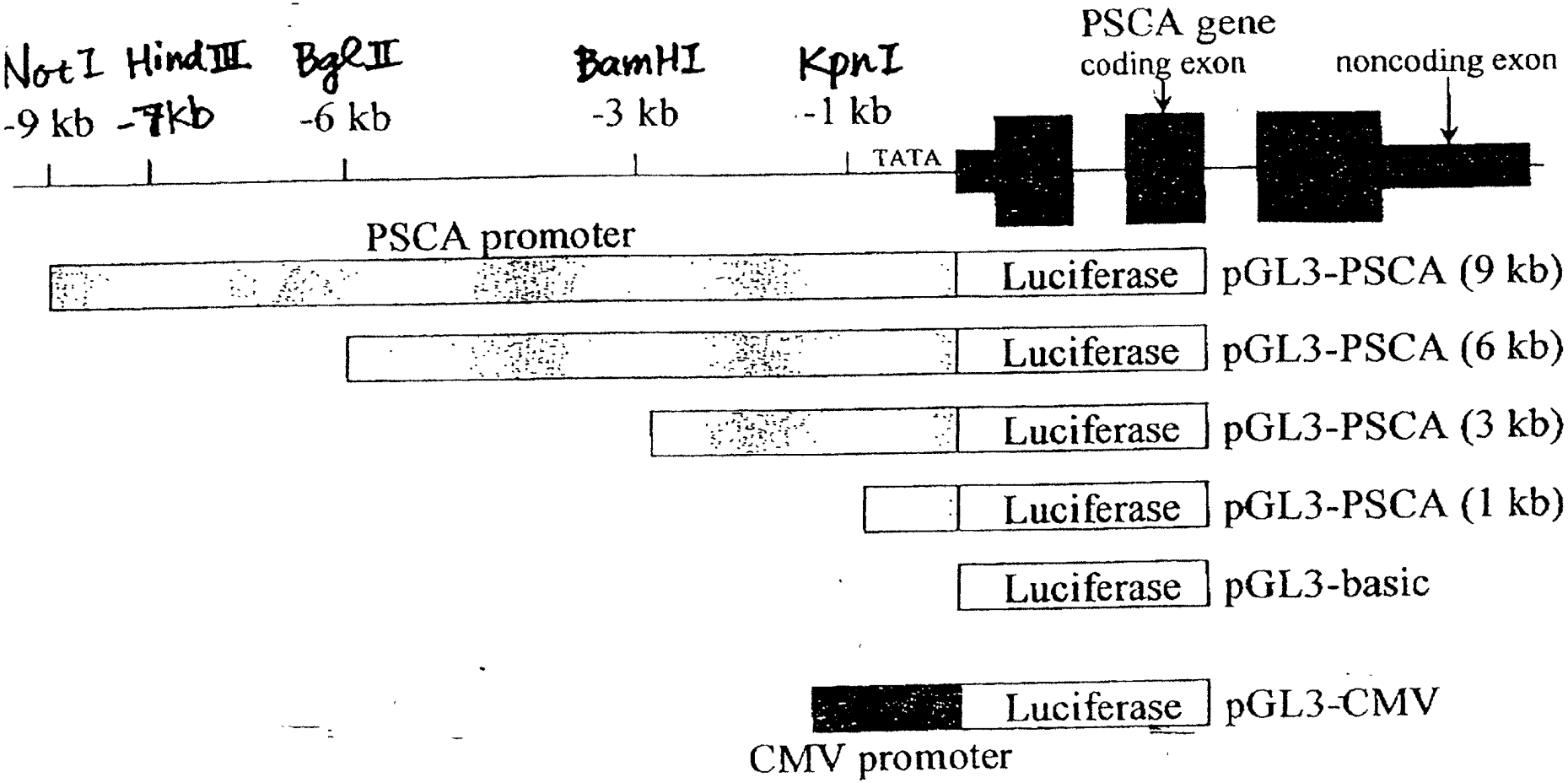


FIGURE 42

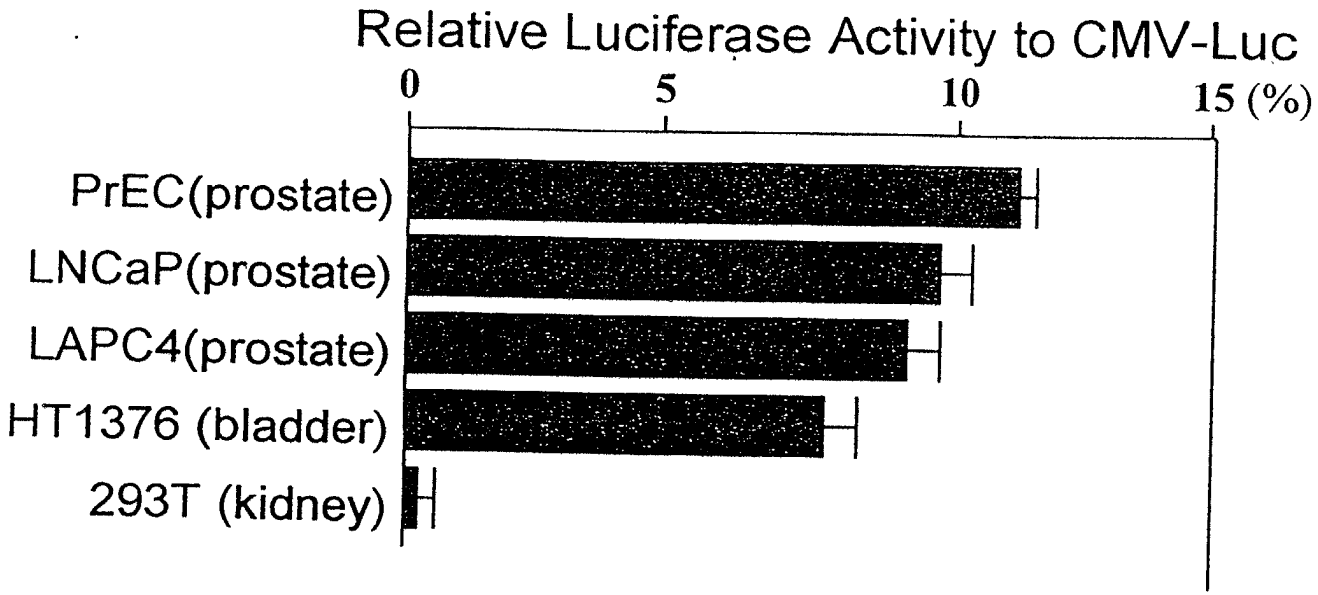


FIGURE 43

Identification of Prostate-Specific Elements Within PSCA Promoter Sequences

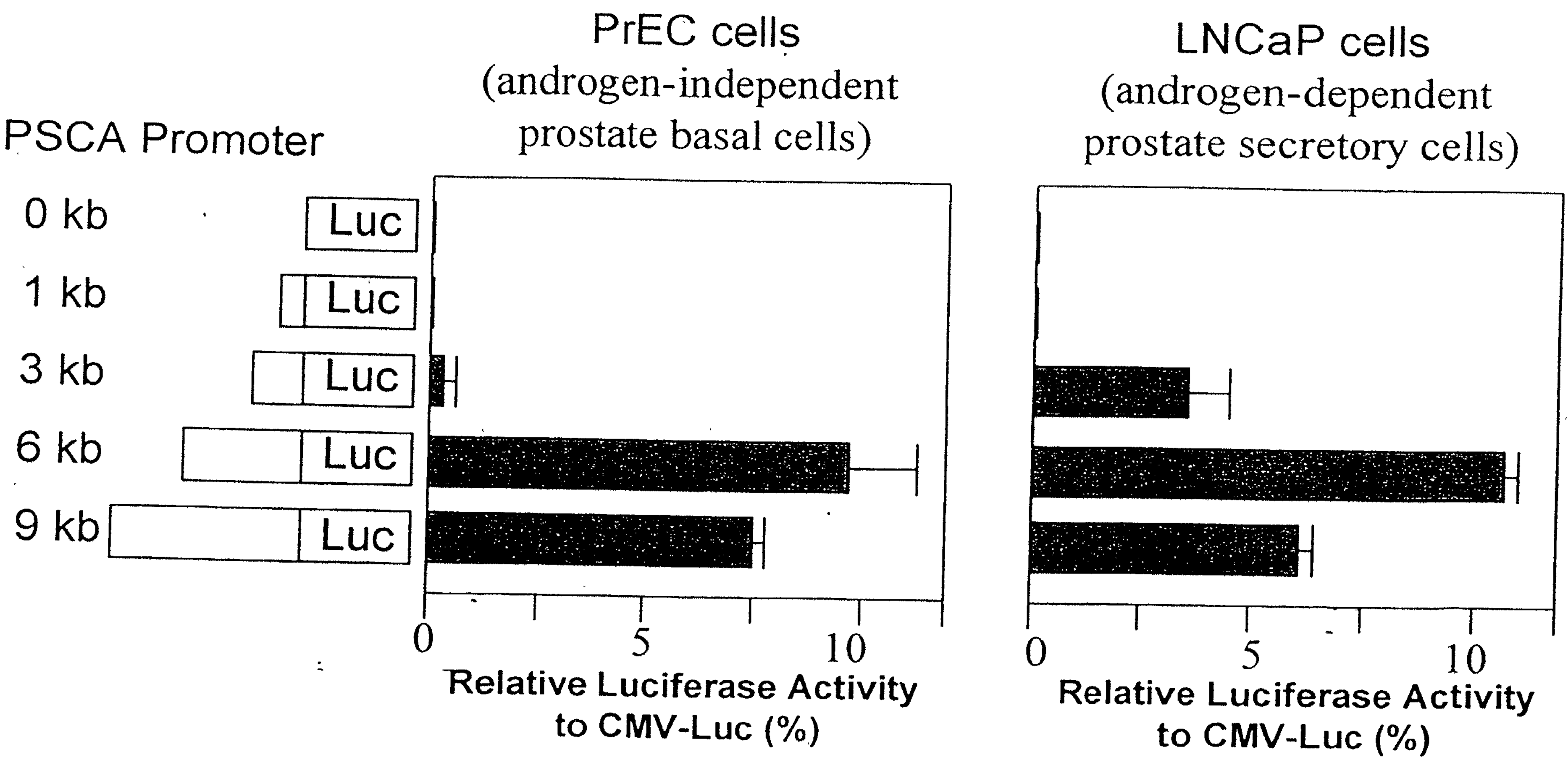
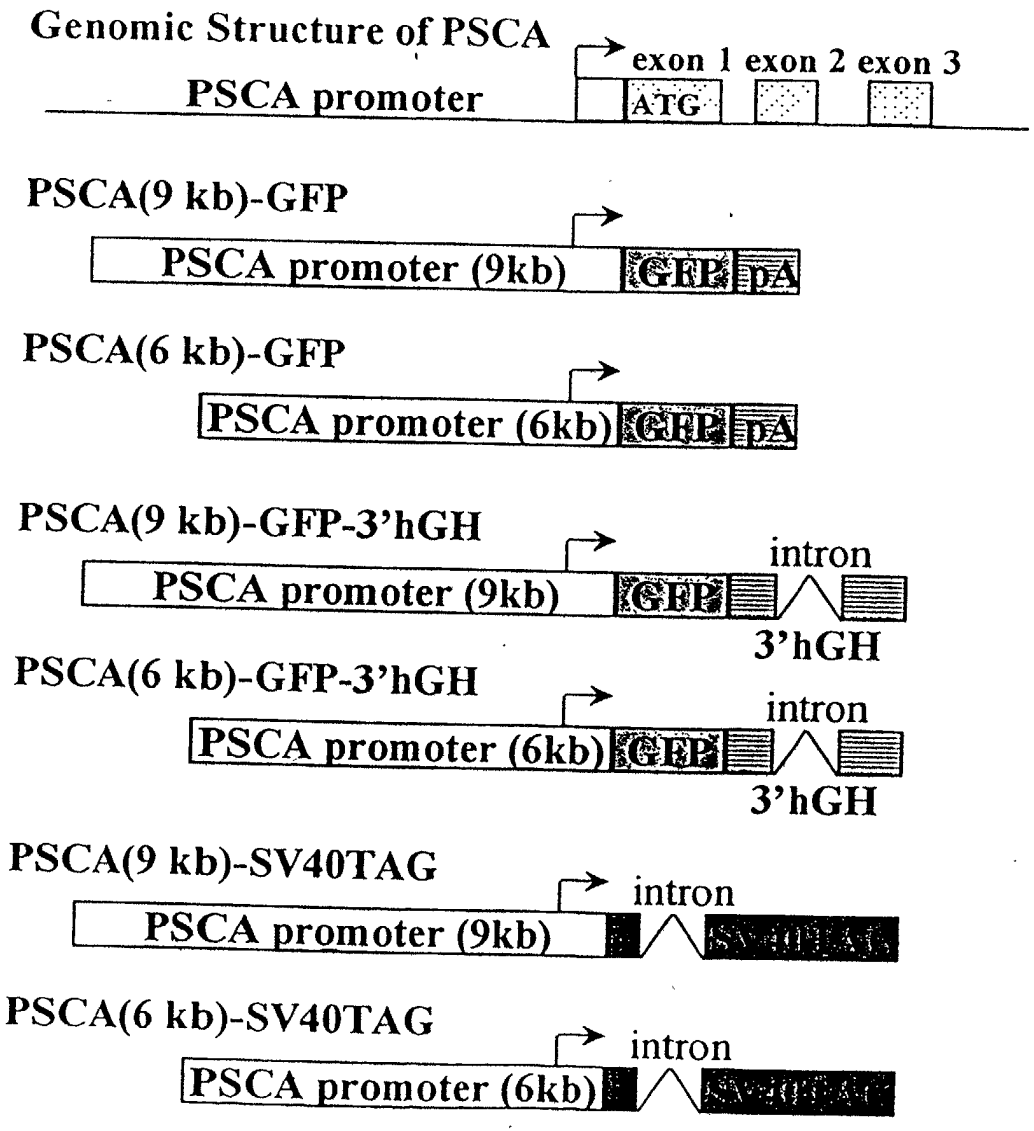


FIGURE 44

Update of Transgenic Mouse Projects



Number of Founders (DNA positive)
2
1
6
8
3
9

FIGURE 45

Whole-mount green fluorescence image

Transgenic Non-transgenic

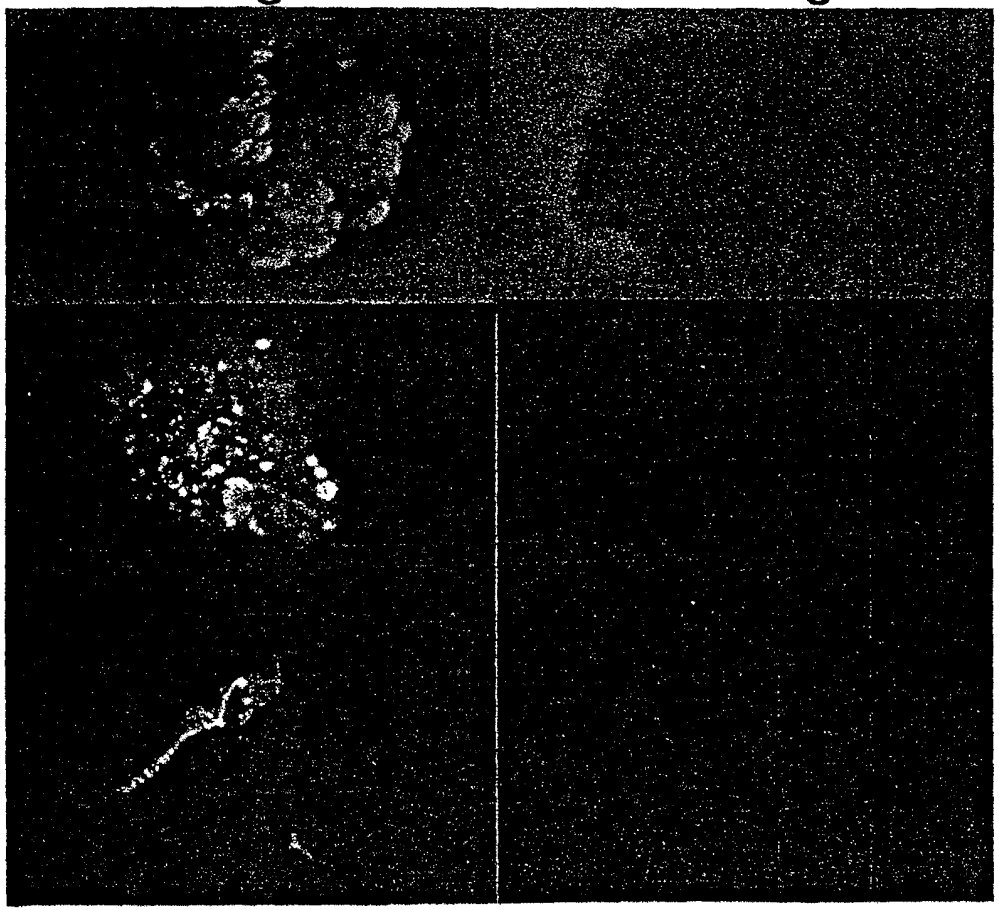
<u>Negative tissues</u>

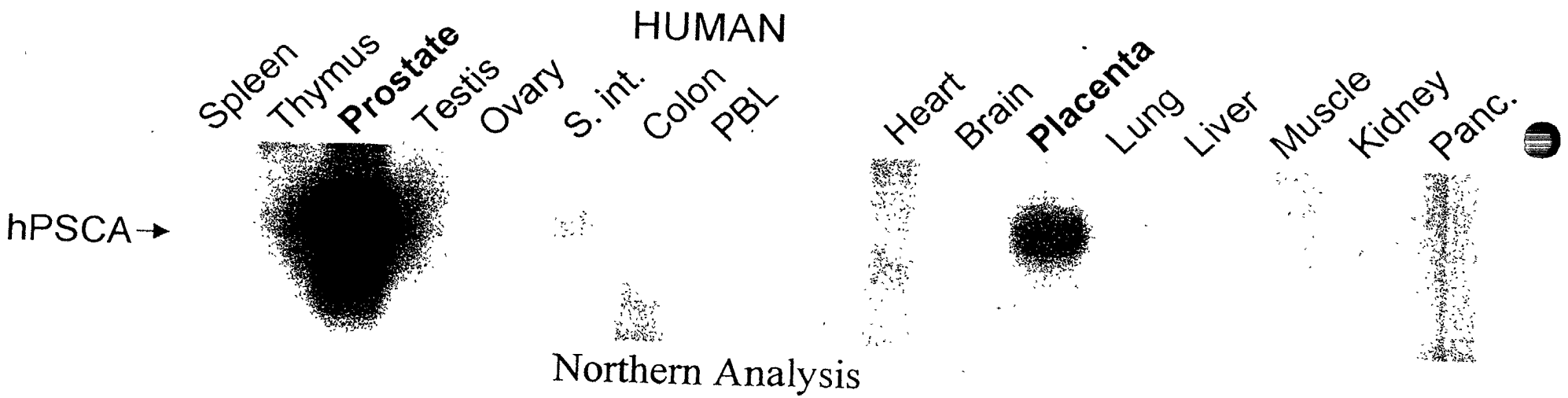
Stomach
Small intestine
Colon
Seminal Vesicle
Urethra
Testis
Liver
Kidney
Lung
Brain
Heart
Skeletal muscle
Ovary
Uterus

Prostate
(A25-106-2)

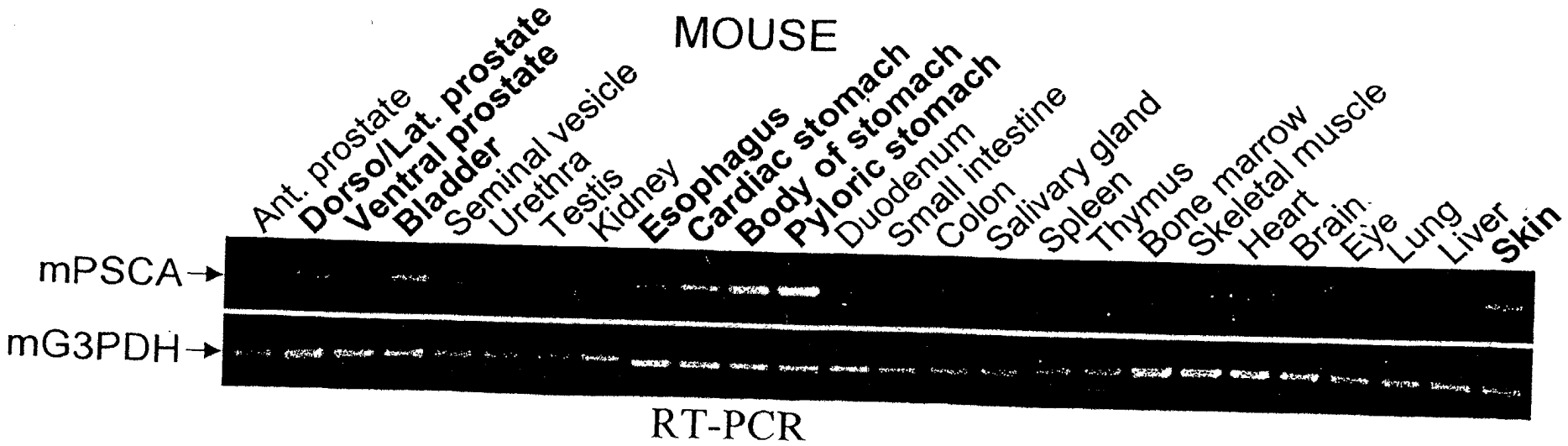
Bladder
(A25-104)

Skin
(A25-106-2)





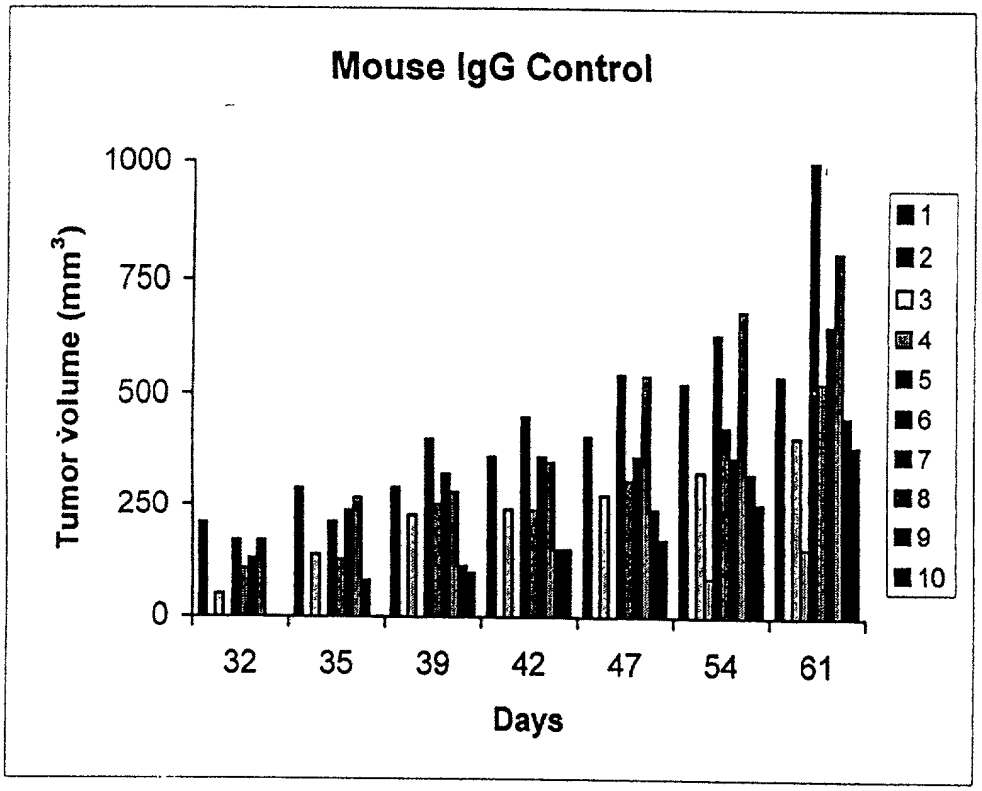
Northern Analysis



RT-PCR

FIGURE 47

FIG. 48


Mouse IgG Control
Tumor volume (mm3)
1000
750
500
250
0
32
35
39
42
47
54
61
Days
1
2
3
4
5
6
7
8
9
10

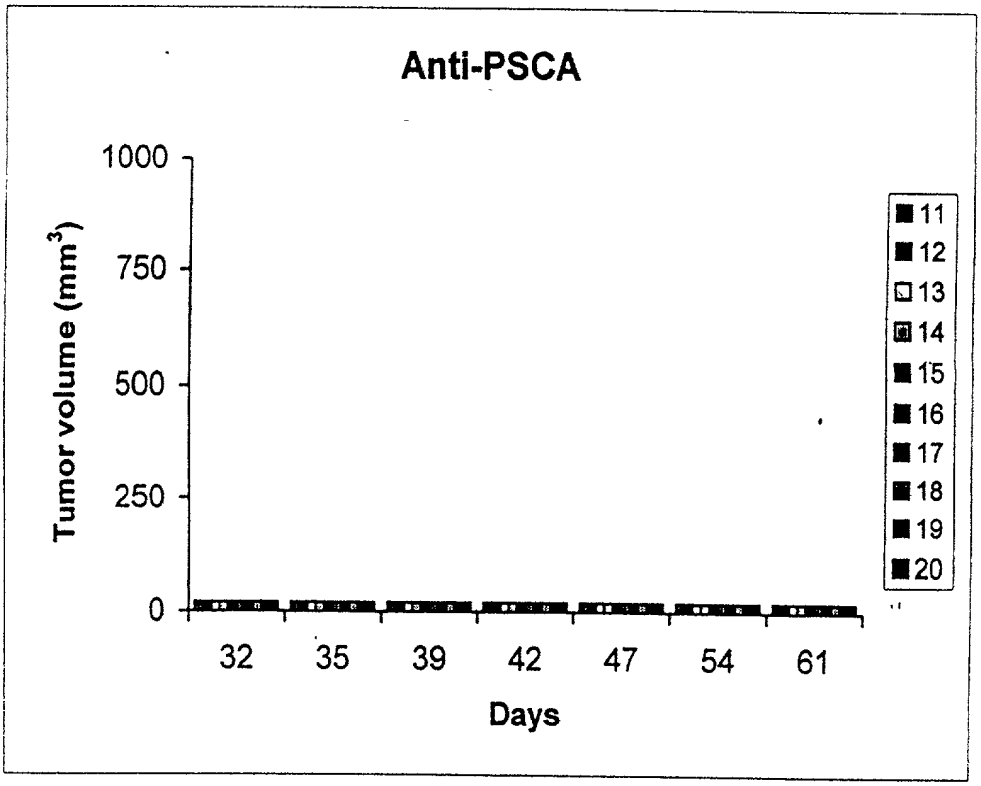

Anti-PSCA
Tumor volume (mm3)
1000
750
500
250
0
32
35
39
42
47
54
61
Days
11
12
13
14
15
16
17
18
19
20

FIG. 49

A

mAb	Isotype	Epitope recognized (OD 450 nm) F (18-98)	N (2-50)	M (46-109)	C (85-123)
1G8	IgG1 k	**1.485**	0.004	**1.273**	0.003
2A2	IgG2a k	**0.973**	**0.631**	0.023	0.010
2H9	IgG1 k	**1.069**	**1.026**	0.002	0.001
3C5	IgG2a k	**1.916**	**1.709**	0.006	0.002
3E6	IgG3 k	**1.609**	0.036	**1.133**	**2.118**
3G3	IgG2a k	**2.805**	**1.731**	0.004	0.000
4A10	IgG2a k	**1.053**	**0.493**	0.000	0.001

B

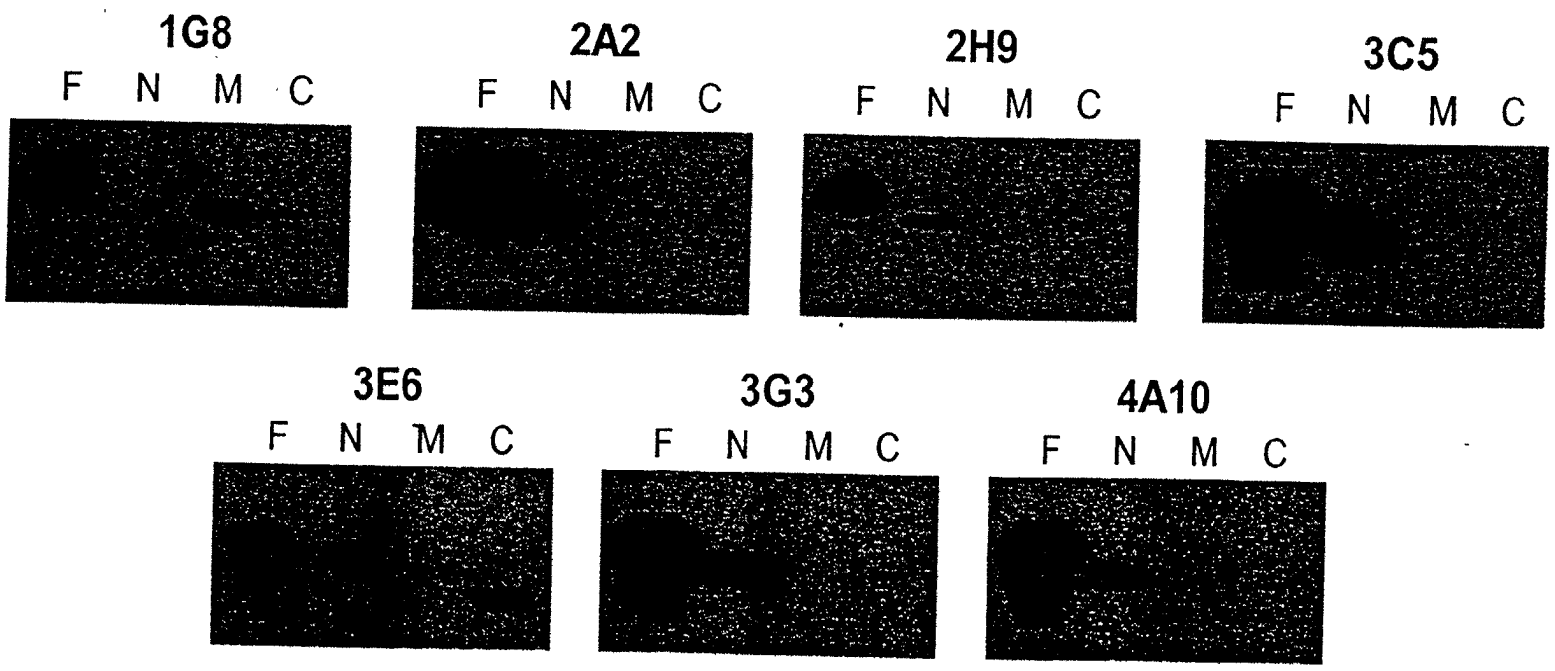
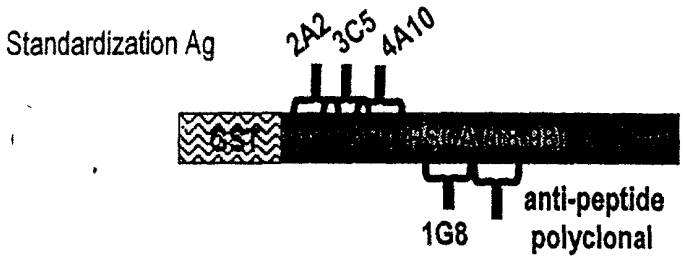
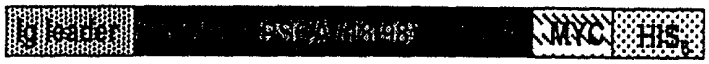


FIG. 50

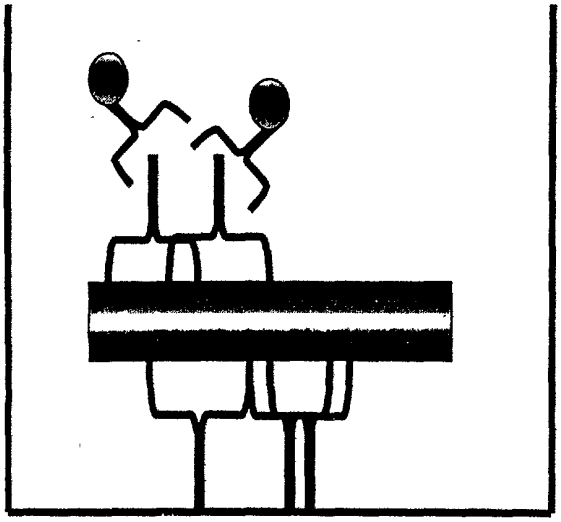
A



Engineered mammalian secreted form



B



Anti-IgG2a HRP

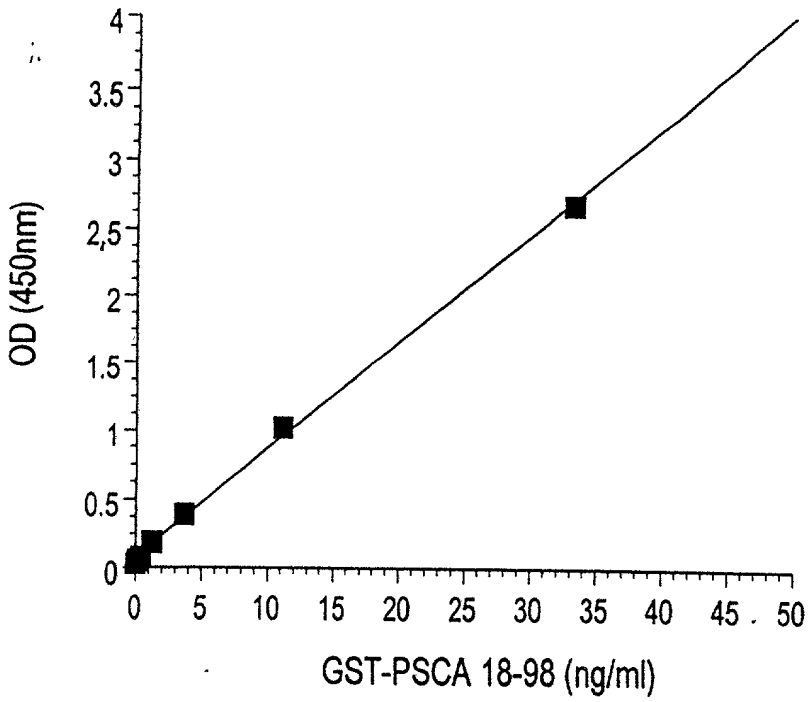
Anti-PSCA mAbs 3C5+4A10+2A2 (IgG2a)

PSCA

Affinity purified anti-peptide polyclonal
+ mAb 1G8 (IgG1)

FIG. 51

A



B

Sample	OD+range (n=2)	ng/ml
vector	0.005+0.001	ND
vector+hu serum	0.004+0.001	ND
secPSCA	2.695+0.031	32.92
secPSCA+hu serum	2.187+0.029	26.55

FIG. 52

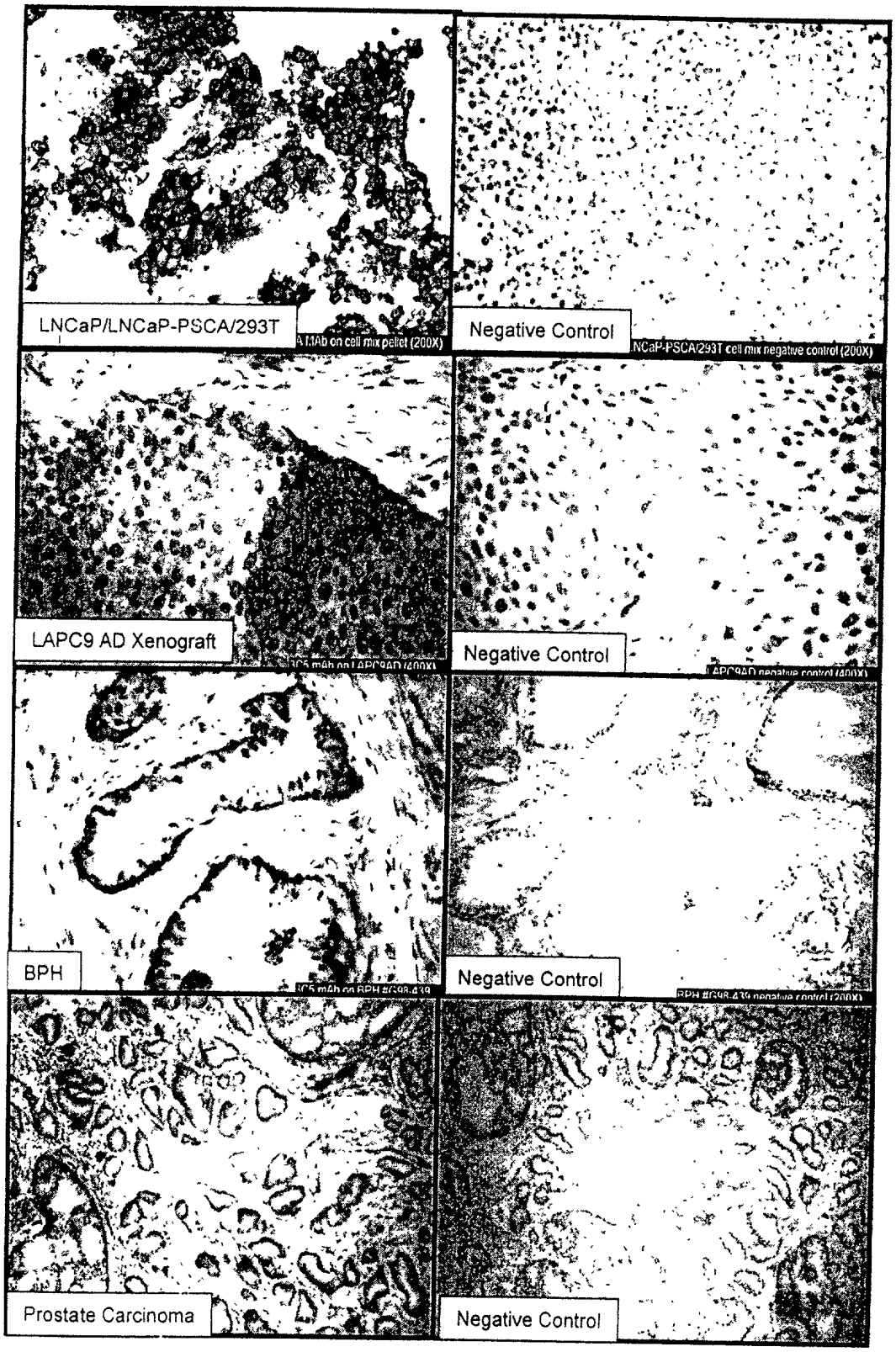
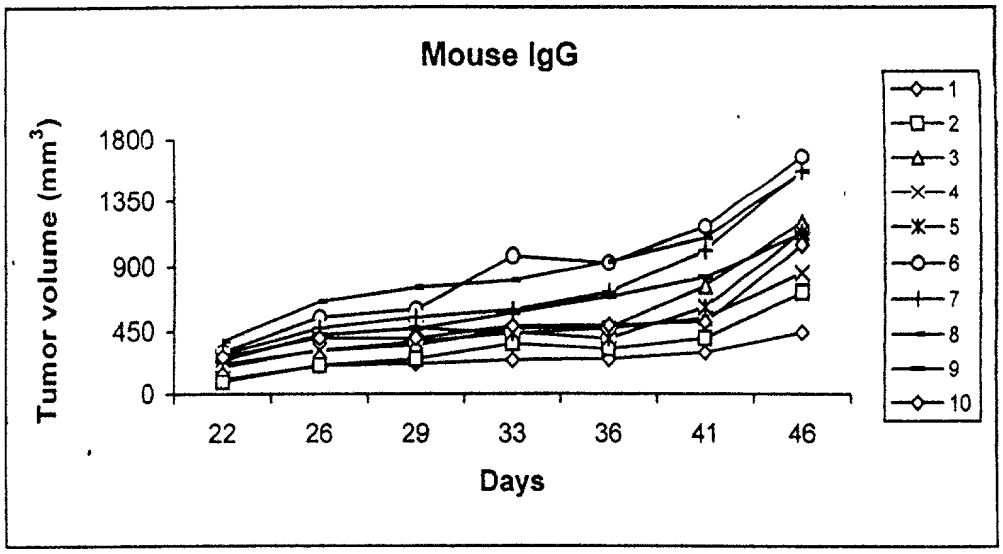
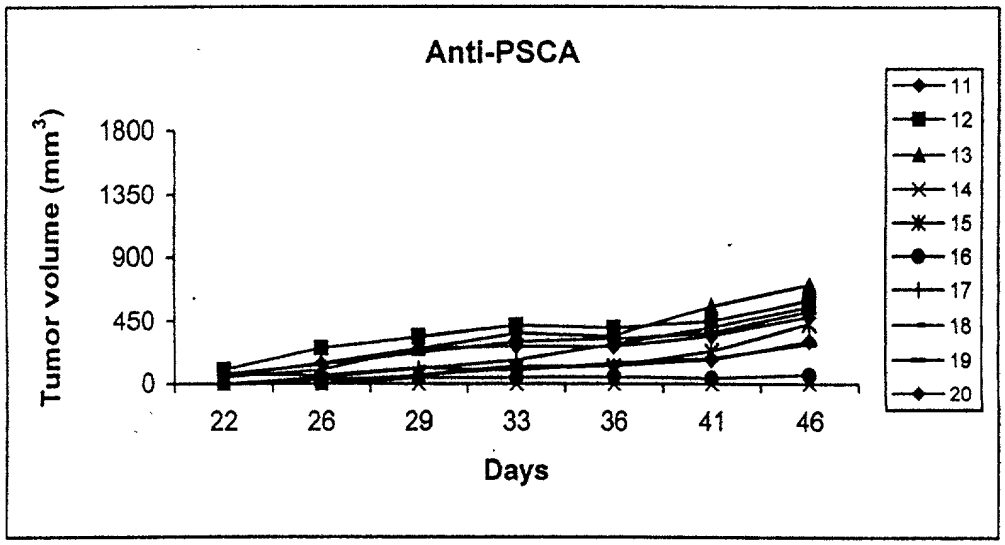

LNCaP/LNCaP-PSCA/293T
Negative Control
LAPC9 AD Xenograft
Negative Control
BPH
Negative Control
Prostate Carcinoma
Negative Control

FIG. 53


Mouse IgG
Tumor volume (mm3)
1800
1350
900
450
0
22
26
29
33
36
41
46
Days
1
2
3
4
5
6
7
8
9
10


Anti-PSCA
Tumor volume (mm3)
1800
1350
900
450
0
22
26
29
33
36
41
46
Days
11
12
13
14
15
16
17
18
19
20

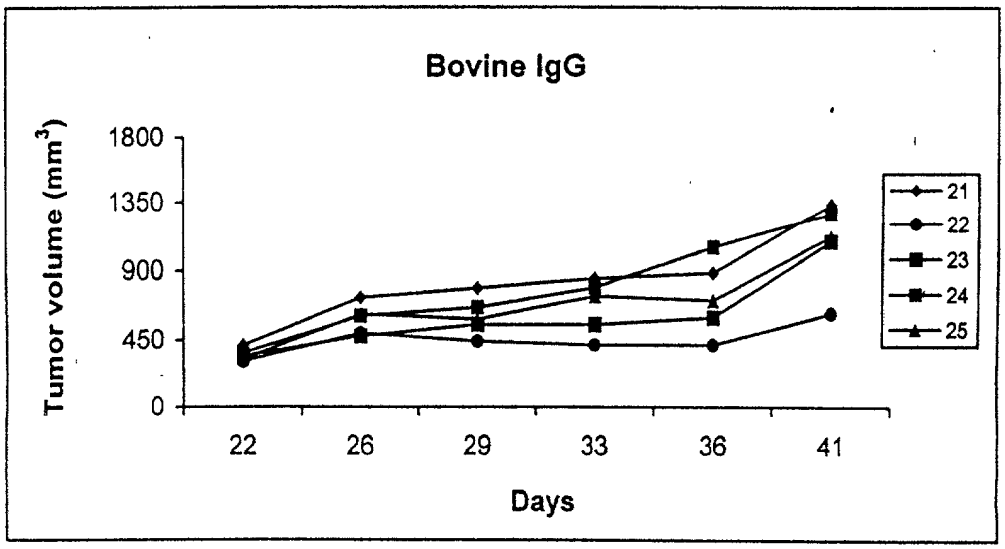
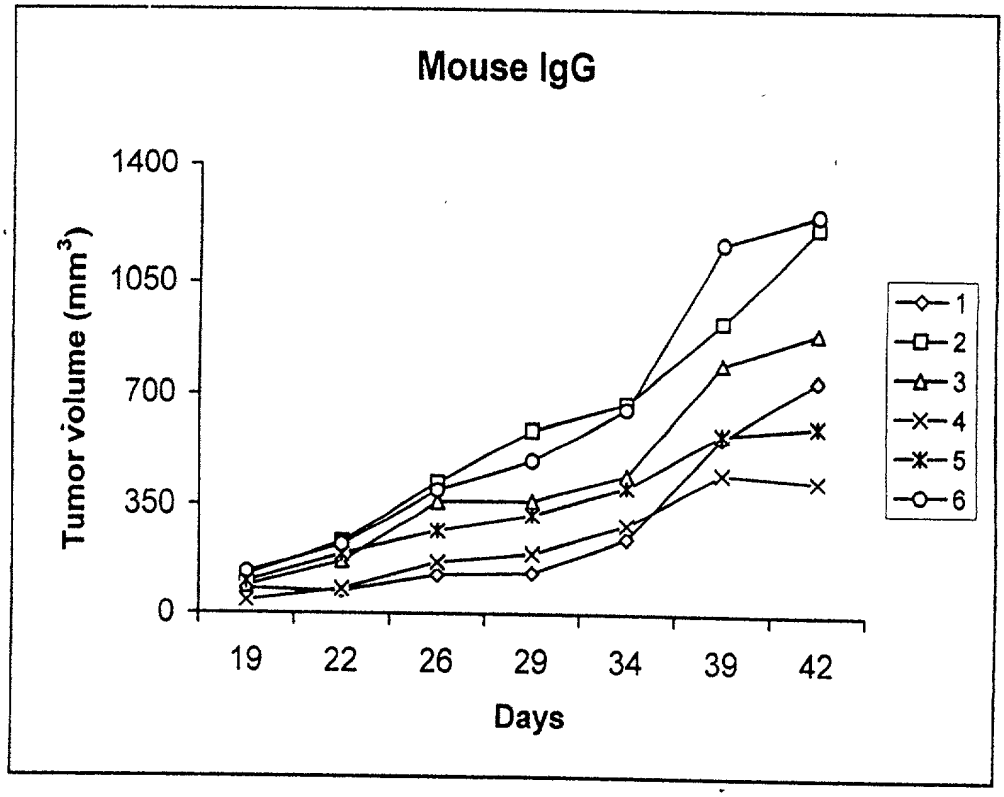

Bovine IgG
Tumor volume (mm3)
1800
1350
900
450
0
22
26
29
33
36
41
Days
21
22
23
24
25

FIG. 54


Mouse IgG
Tumor volume (mm3)
1400
1050
700
350
0
19
22
26
29
34
39
42
Days
1
2
3
4
5
6

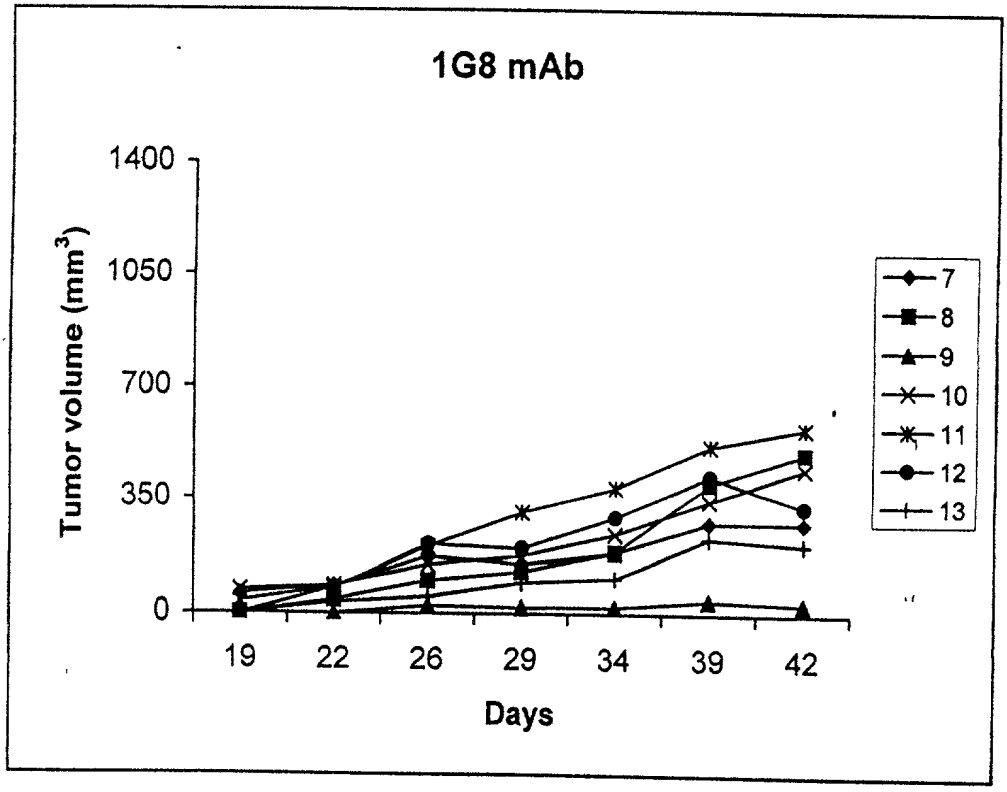
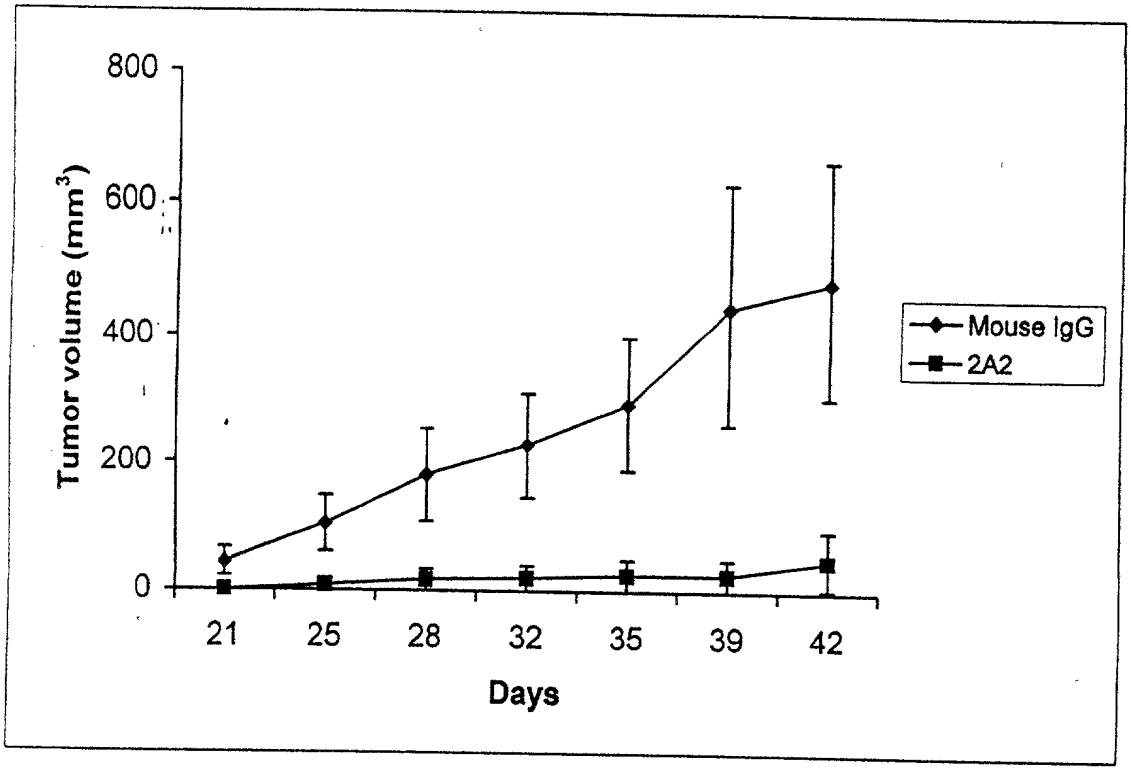

1G8 mAb
Tumor volume (mm3)
1400
1050
700
350
0
19
22
26
29
34
39
42
Days
7
8
9
10
11
12
13

FIG. 55


800
600
400
200
0
Tumor volume (mm3)
21
25
28
32
35
39
42
Days
Mouse IgG
2A2

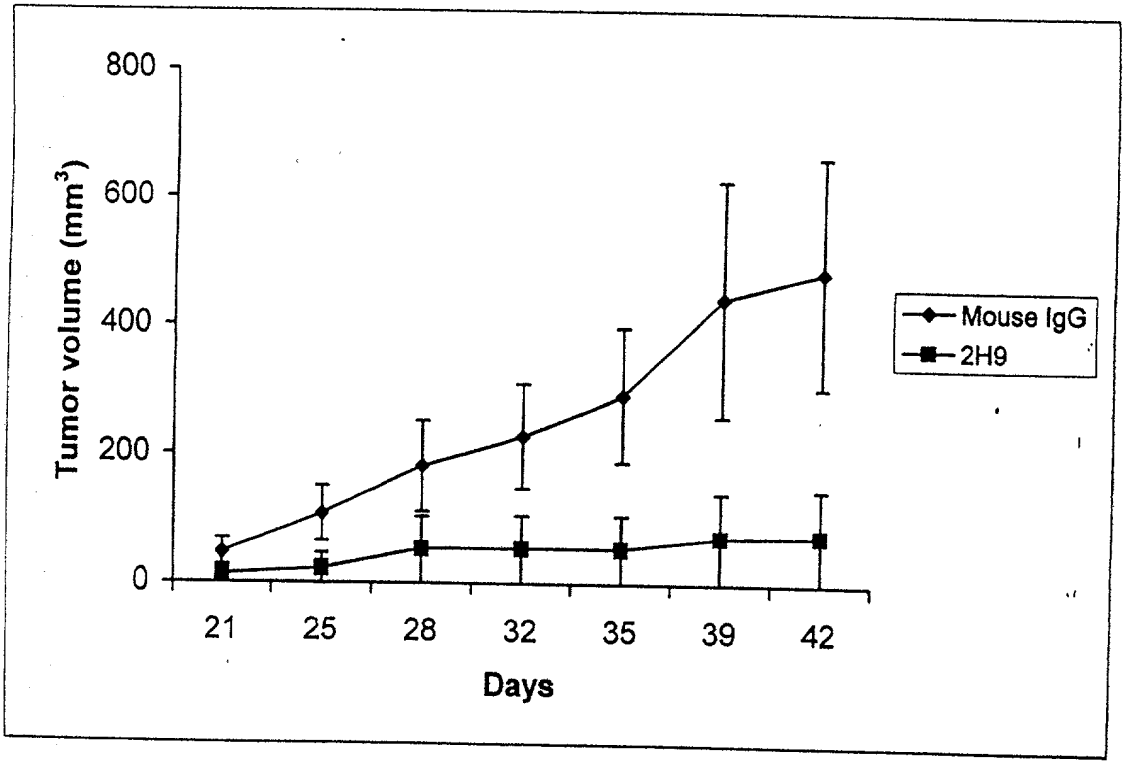
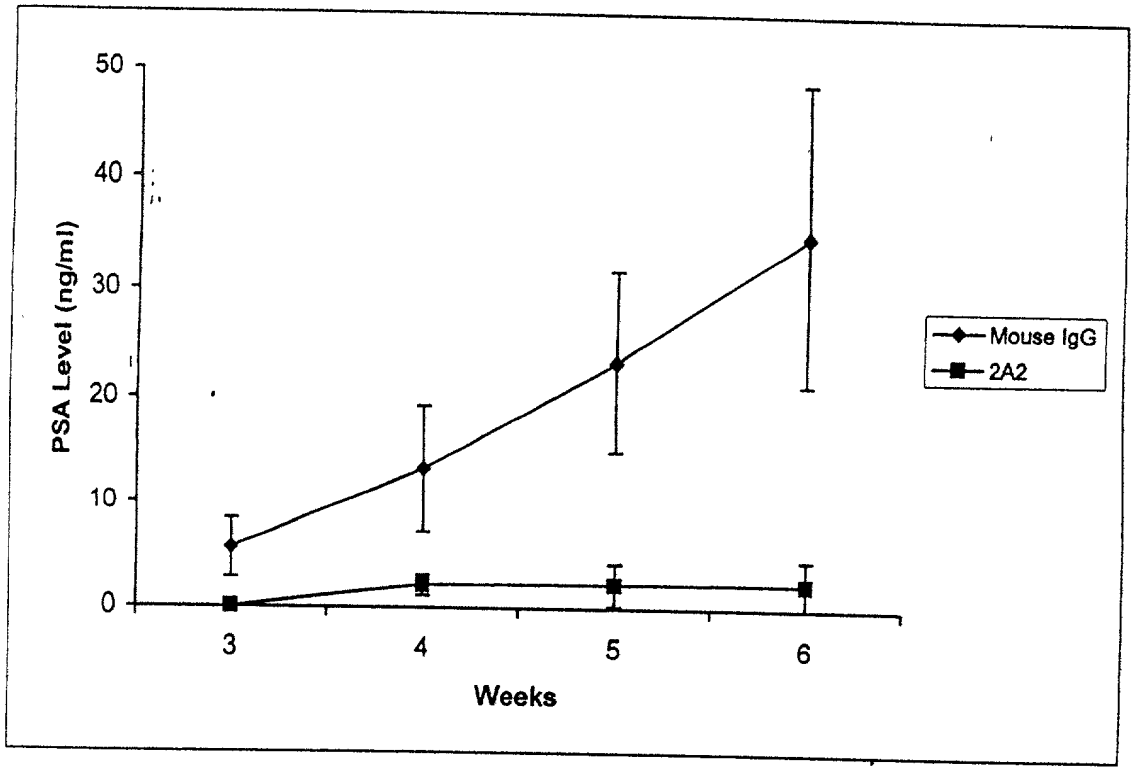

800
600
400
200
0
Tumor volume (mm3)
21
25
28
32
35
39
42
Days
Mouse IgG
2H9

FIG. 56


50
40
30
20
10
0
PSA Level (ng/ml)
3
4
5
6
Weeks
Mouse IgG
2A2

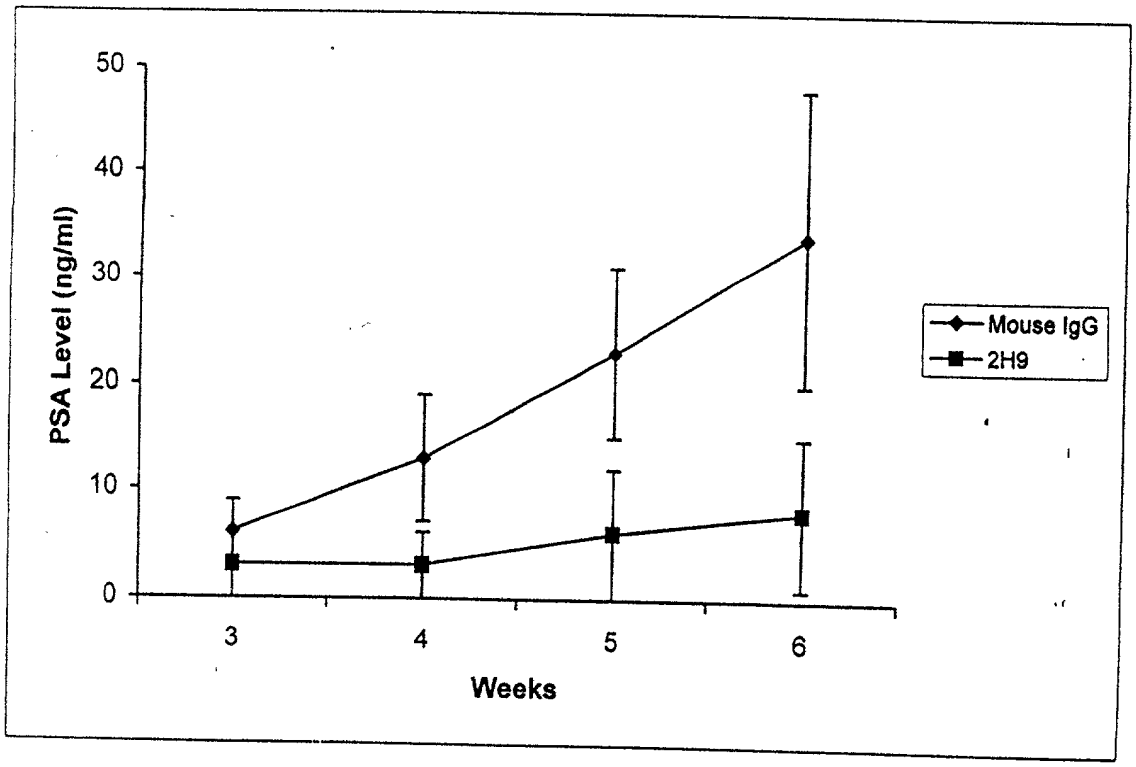

50
40
30
20
10
0
PSA Level (ng/ml)
3
4
5
6
Weeks
Mouse IgG
2H9

FIG. 57

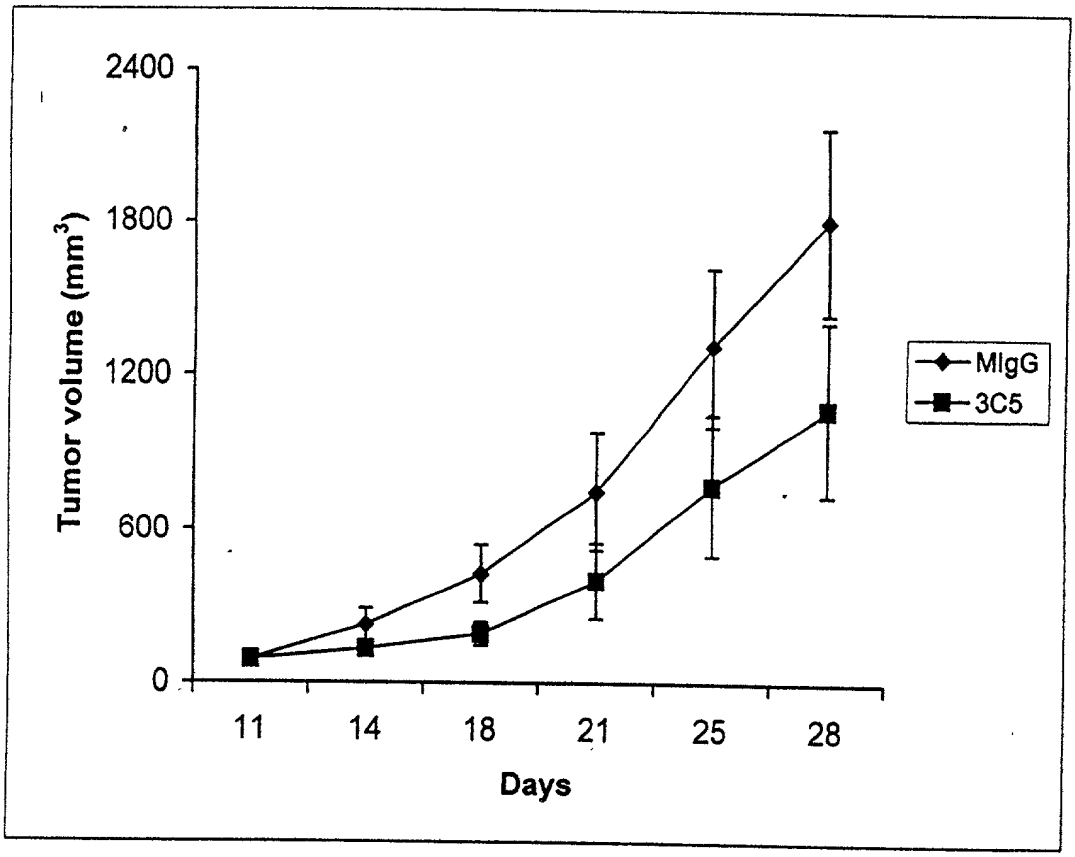

2400
1800
1200
600
0
Tumor volume (mm3)
11
14
18
21
25
28
Days
MIgG
3C5

FIG. 58

```
TGCTTCTTCCTGATGGCAGTGGTTATAGGAGTCAATTCAGAGGTTCAGCTGCAGCAGTCT  60
 C  F  F  L  M  A  V  V  I  G  V  N  S  E  V  Q  L  Q  Q  S   20

                                                    ┌──────
GGGGCAGAACTTGTGAGGTCAGGGGCCTCAGTCAAGTTGTCCTGCACAGCTTCTGGCTTC 120
 G  A  E  L  V  R  S  G  A  S  V  K  L  S  C  T  A  S  G  F   40

        ──────── CDR1 ────────┐
AACATTAAAGACTACTATATACACTGGGTGAATCAGAGGCCTGACCAGGGCCTGGAGTGG 180
 N  I  K  D  Y  Y  I  H  W  V  N  Q  R  P  D  Q  G  L  E  W   60

          ┌──────────────────────── CDR2 ──────────────────────┐
ATTGGATGGATTGATCCTGAGAATGGTGACACTGAATTTGTCCCGAAGTTCCAGGGCAAG 240
 I  G  W  I  D  P  E  N  G  D  T  E  F  V  P  K  F  Q  G  K   80

GCCACTATGACTGCAGACATTTTCTCCAACACAGCCTACCTGCACCTCAGCAGCCTGACA 300
 A  T  M  T  A  D  I  F  S  N  T  A  Y  L  H  L  S  S  L  T  100

                                          ┌ CDR3 ┐
TCTGAAGACACTGCCGTCTATTACTGTAAAACGGGGGGTTTCTGGGGCCAAGGGACTCTG 360
 S  E  D  T  A  V  Y  Y  C  K  T  G  G  F  W  G  Q  G  T  L  120

GTCACTGTCTCTGCAGCCAAAACGACACCCCCATCTGTCTATCCACTG
 V  T  V  S  A  A  K  T  T  P  P  S  V  Y  P  L
```

FIG. 59

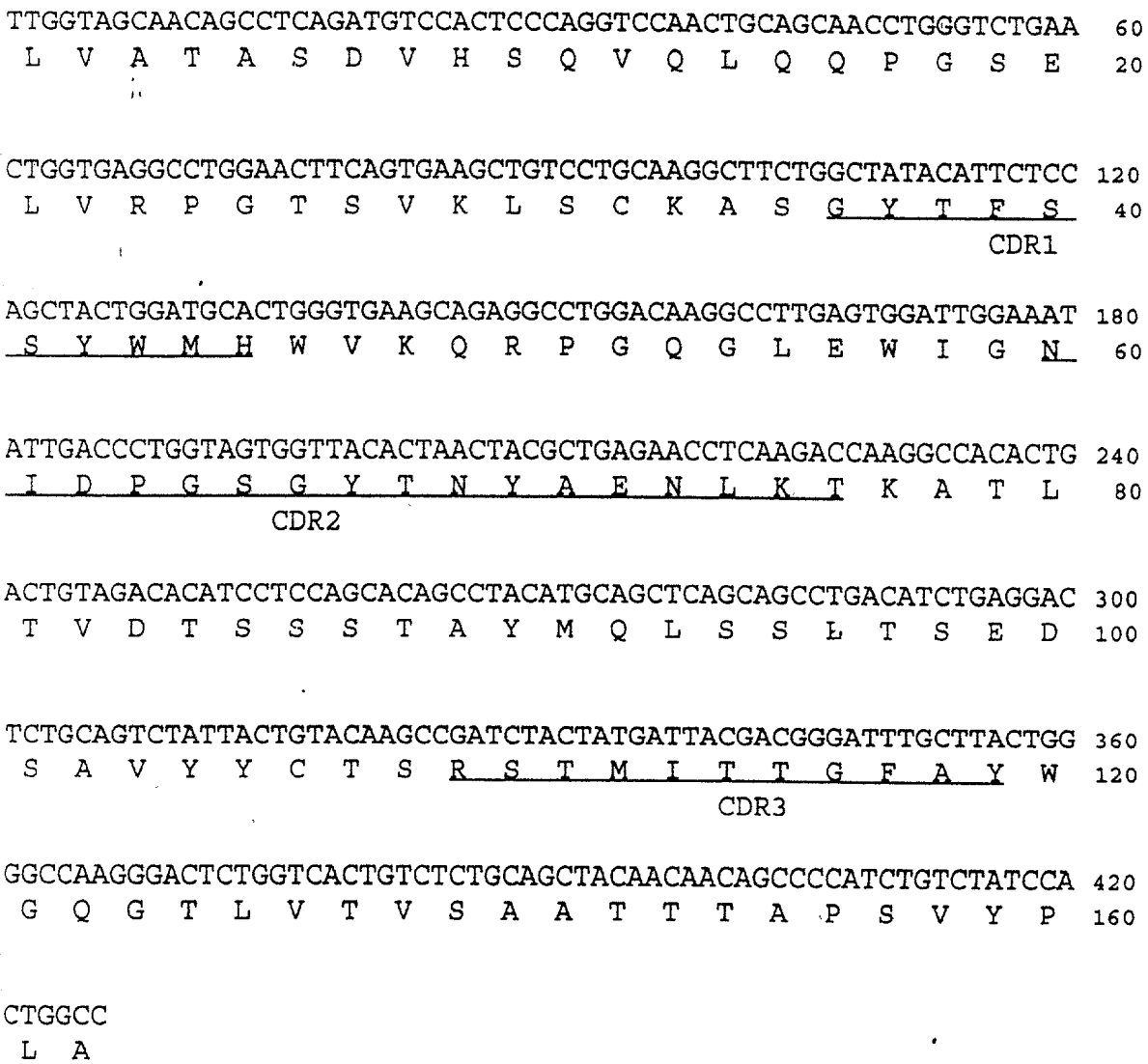


FIG. 60

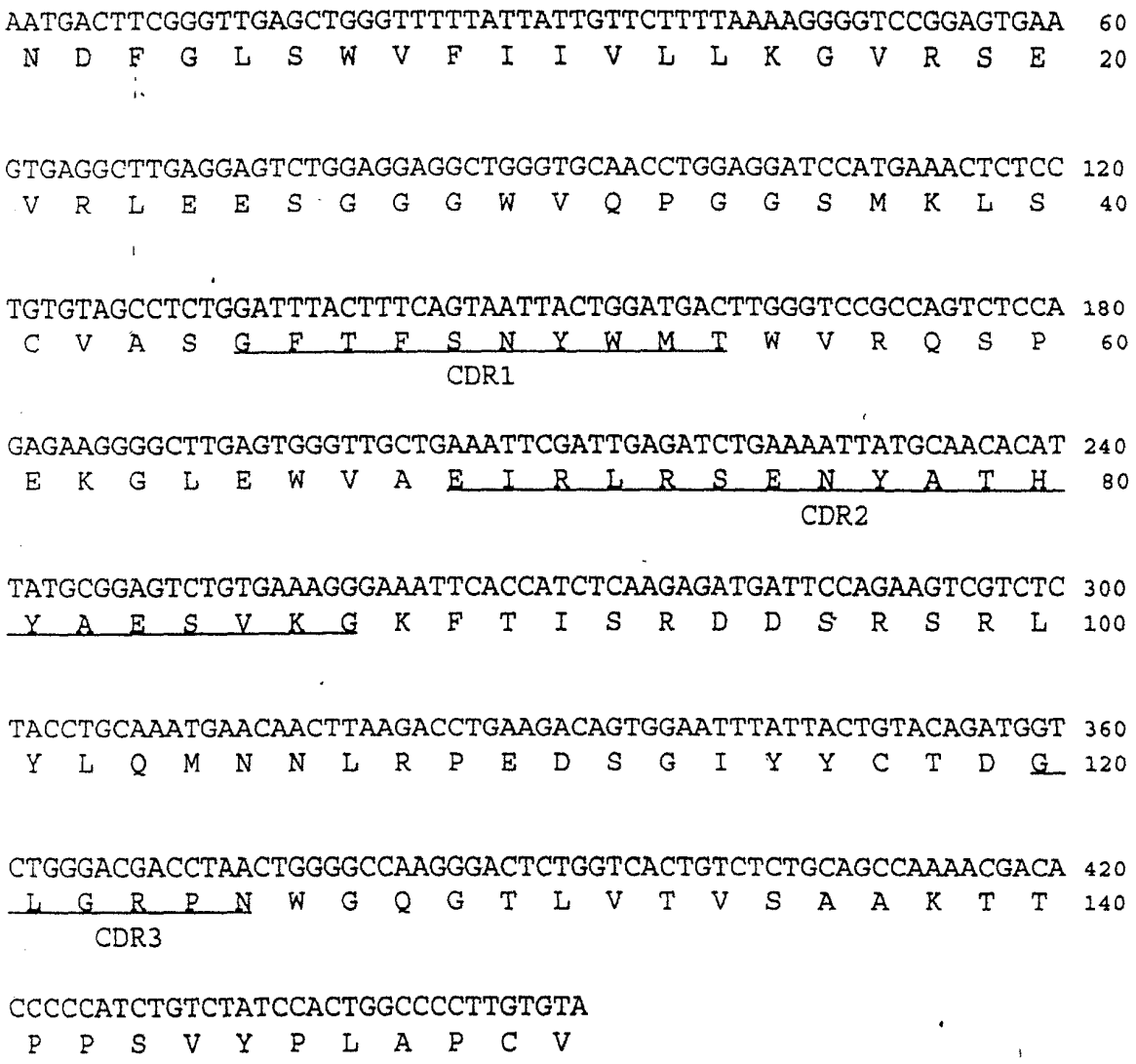

AATGACTTCGGGTTGAGCTGGGTTTTTATTATTGTTCTTTTAAAAGGGGTCCGGAGTGAA 60
N D F G L S W V F I I V L L K G V R S E 20
GTGAGGCTTGAGGAGTCTGGAGGAGGCTGGGTGCAACCTGGAGGATCCATGAAACTCTCC 120
V R L E E S G G G W V Q P G G S M K L S 40
TGTGTAGCCTCTGGATTTACTTTCAGTAATTACTGGATGACTTGGGTCCGCCAGTCTCCA 180
C V A S G F T F S N Y W M T W V R Q S P 60
CDR1
GAGAAGGGGCTTGAGTGGGTTGCTGAAATTCGATTGAGATCTGAAAATTATGCAACACAT 240
E K G L E W V A E I R L R S E N Y A T H 80
CDR2
TATGCGGAGTCTGTGAAAGGGAAATTCACCATCTCAAGAGATGATTCCAGAAGTCGTCTC 300
Y A E S V K G K F T I S R D D S R S R L 100
TACCTGCAAATGAACAACTTAAGACCTGAAGACAGTGGAATTTATTACTGTACAGATGGT 360
Y L Q M N N L R P E D S G I Y Y C T D G 120
CTGGGACGACCTAACTGGGGCCAAGGGACTCTGGTCACTGTCTCTGCAGCCAAAACGACA 420
L G R P N W G Q G T L V T V S A A K T T 140
CDR3
CCCCCATCTGTCTATCCACTGGCCCCTTGTGTA
P P S V Y P L A P C V

FIG. 61

CDR1 Comparisons

1G8	IgG_{1k}	Middle	G F N I K D Y Y I H
2H9	IgG_{1k}	N-Term.	G F T F S N Y W M T
4A10	IgG_{2ak}	N-Term.	G Y T F S S Y W M H

CDR2 Comparisons

1G8	IgG_{1k}	W I D P E N G D T E F V P K F Q G
2H9	IgG_{1k}	E I R L R S E N Y A T H Y A E S V K G
4A10	IgG_{2ak}	N I D P G S G Y T N Y A E N L K T

CDR3 Comparisons

1G8	IgG_{1k}	G G F
2H9	IgG_{1k}	L G R P N
4A10	IgG_{2ak}	R S T M I T T G F A Y

FIG. 62

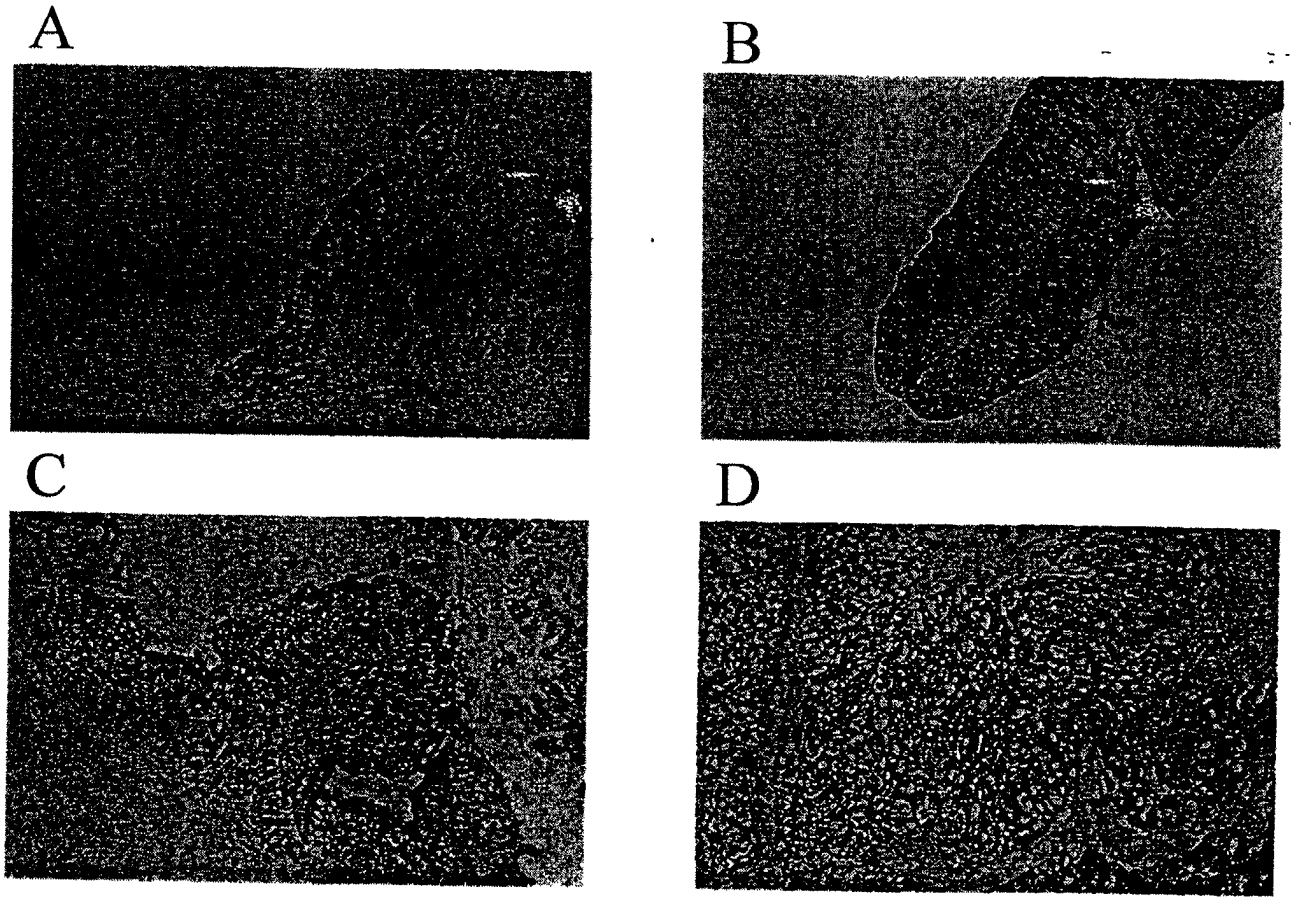
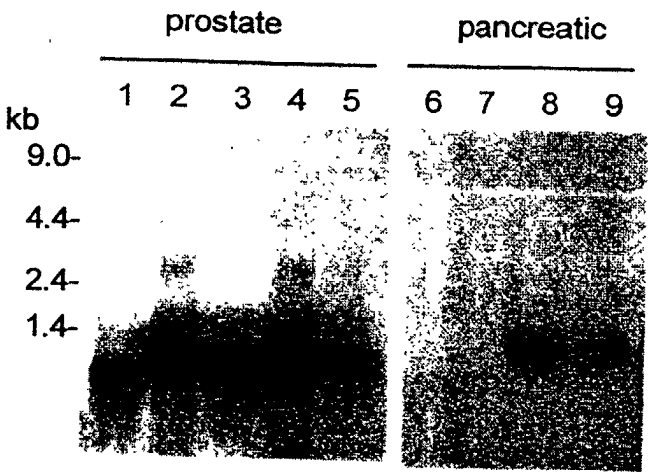
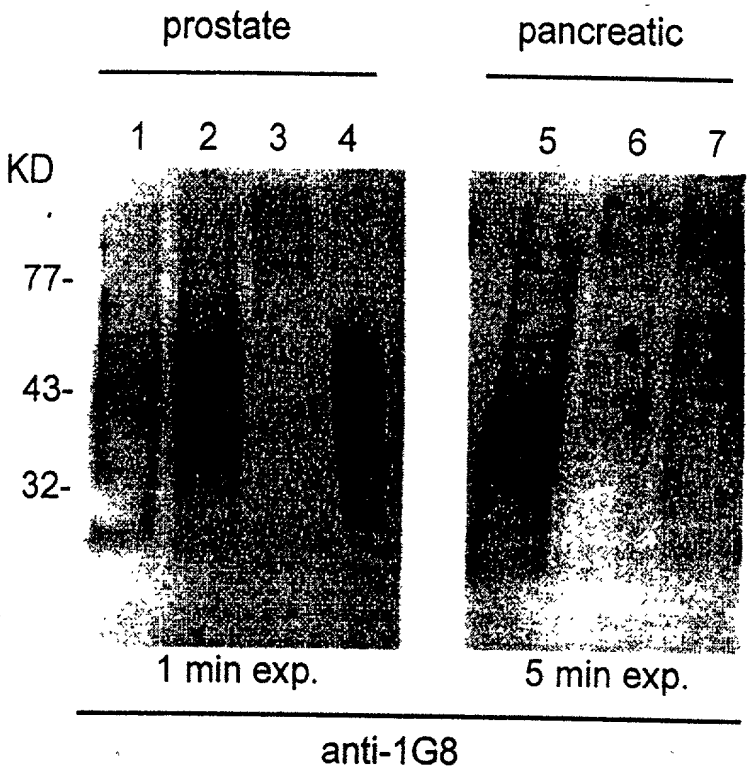


FIG. 63



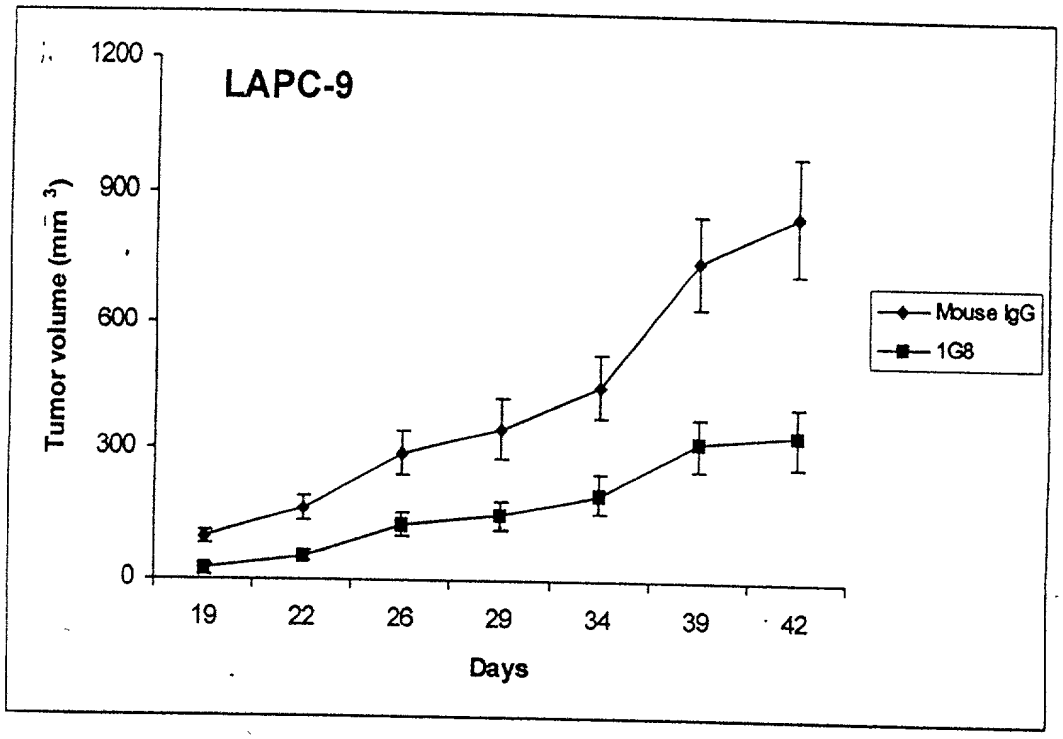
1. Prostate
2. LAPC-4 AD
3. LAPC-4 AI
4. LAPC-9 AD
5. LAPC-9 AI
6. PANC-1
7. BxPC-3
8. HPAC
9. Capan-1

FIG. 64



1. LAPC-4 AD
2. LAPC-9 AI
3. LNCaP
4. LNCaP-PSCA

5. HPAC
6. Capan-1
7. ASPC-1


LAPC-9
Tumor volume (mm 3)
1200
900
600
300
0
19
22
26
29
34
39
42
Days
Mouse IgG
1G8

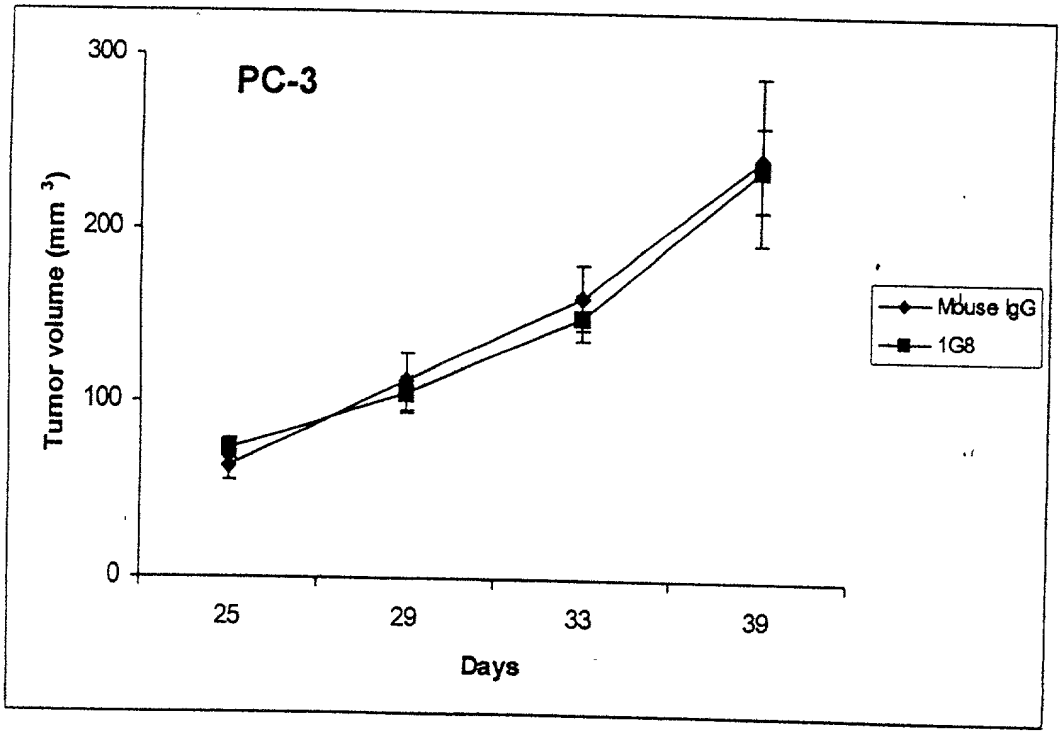
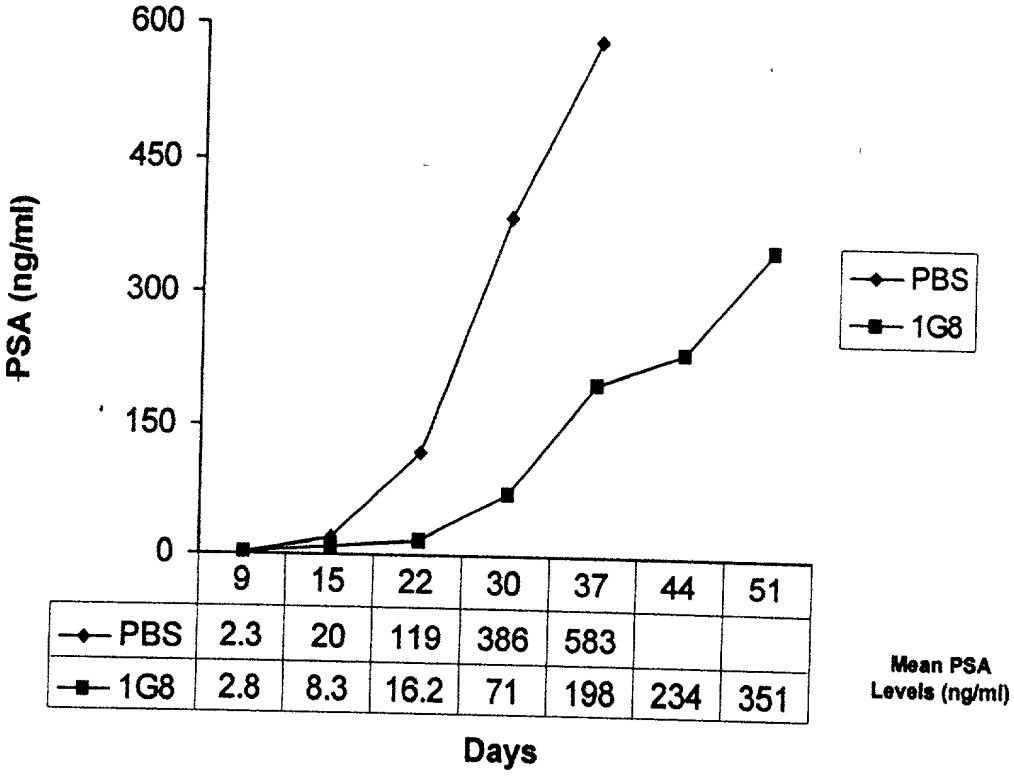

PC-3
Tumor volume (mm 3)
300
200
100
0
25
29
33
39
Days
Mouse IgG
1G8

FIGURE 65

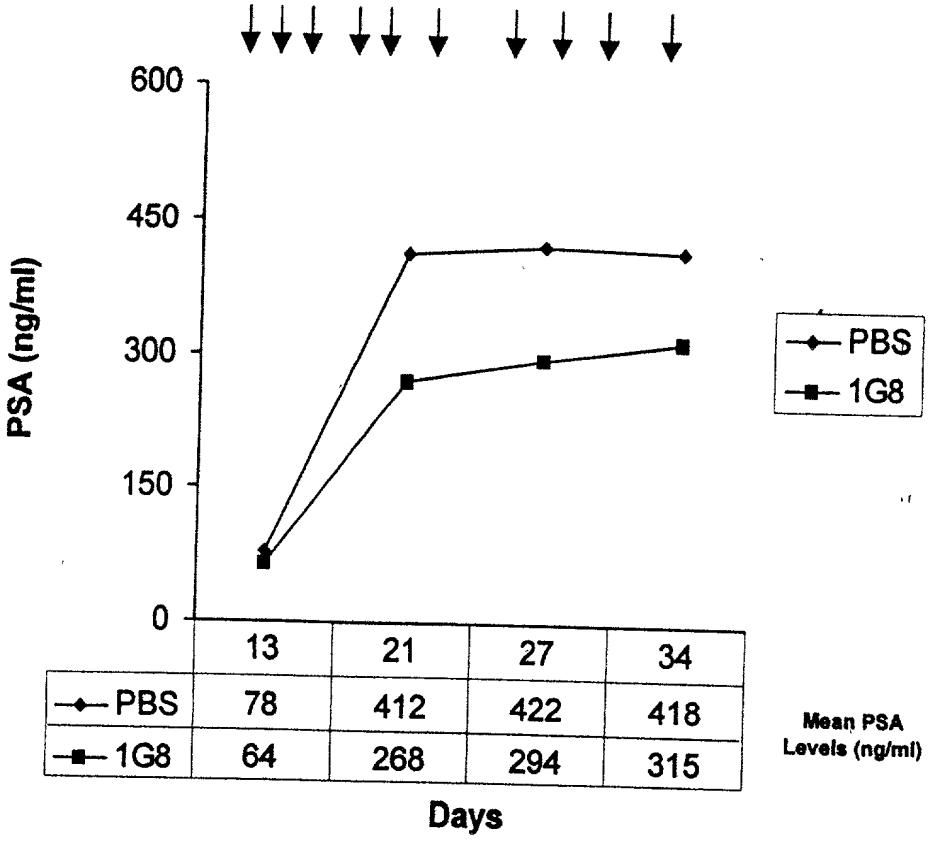
A)



	9	15	22	30	37	44	51
PBS	2.3	20	119	386	583		
1G8	2.8	8.3	16.2	71	198	234	351

Mean PSA Levels (ng/ml)

B)

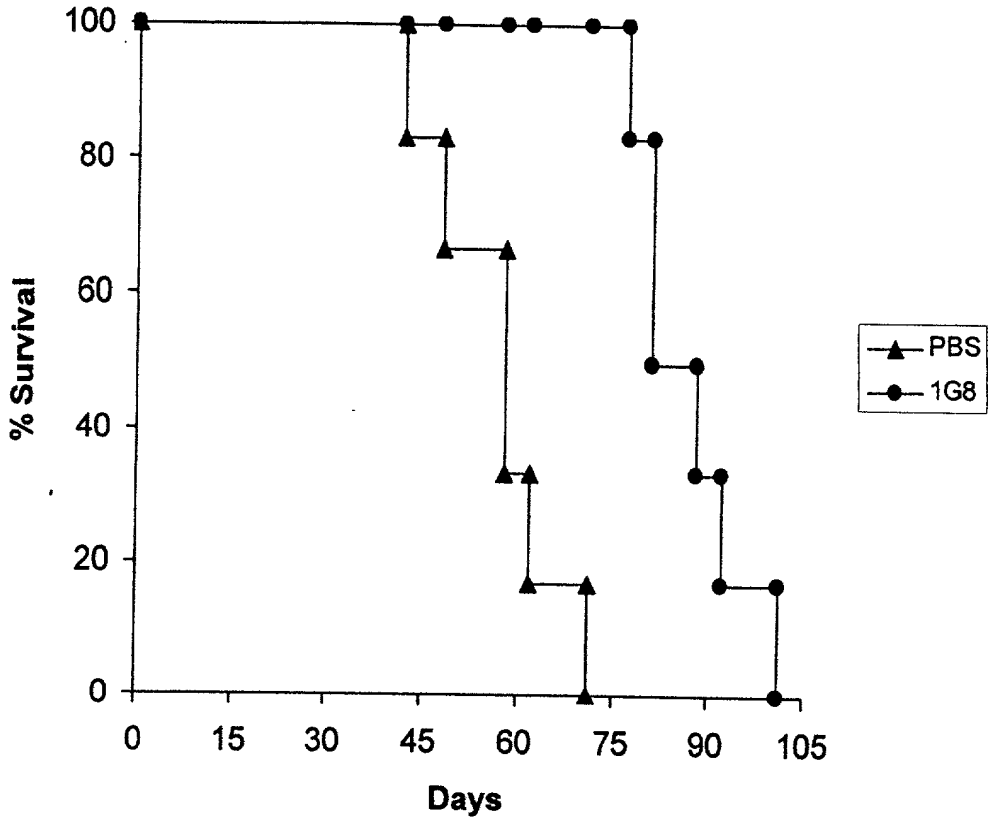


	13	21	27	34
PBS	78	412	422	418
1G8	64	268	294	315

Mean PSA Levels (ng/ml)

Figure 66

A)


100
80
60
40
20
0
% Survival
0
15
30
45
60
75
90
105
Days
PBS
1G8

B)

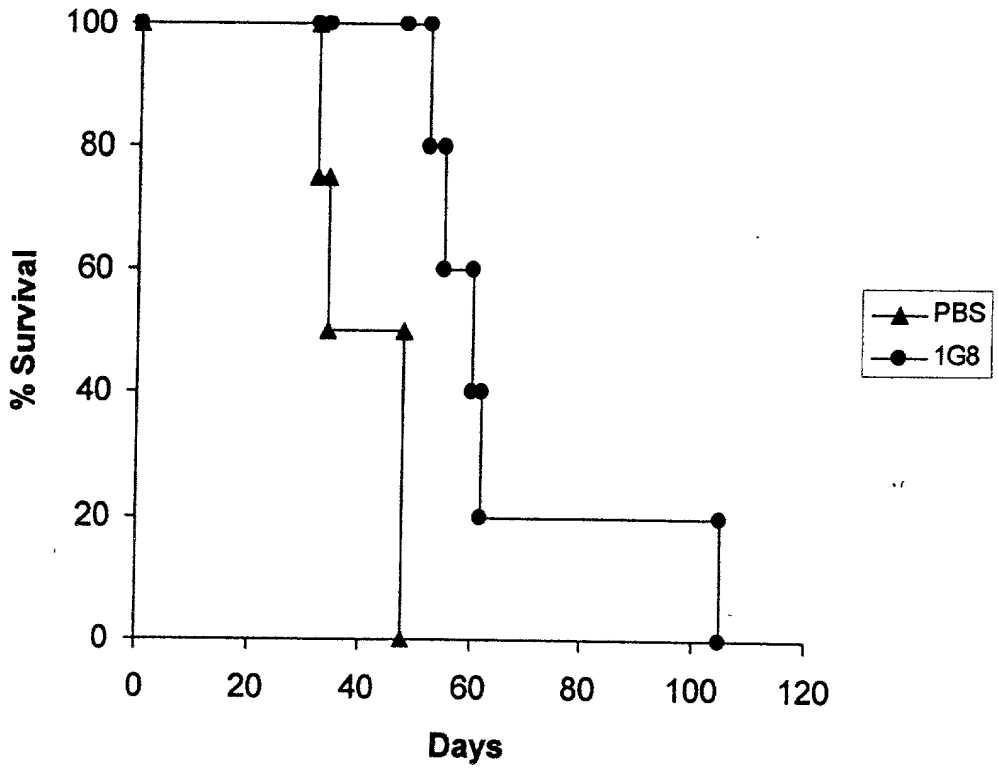
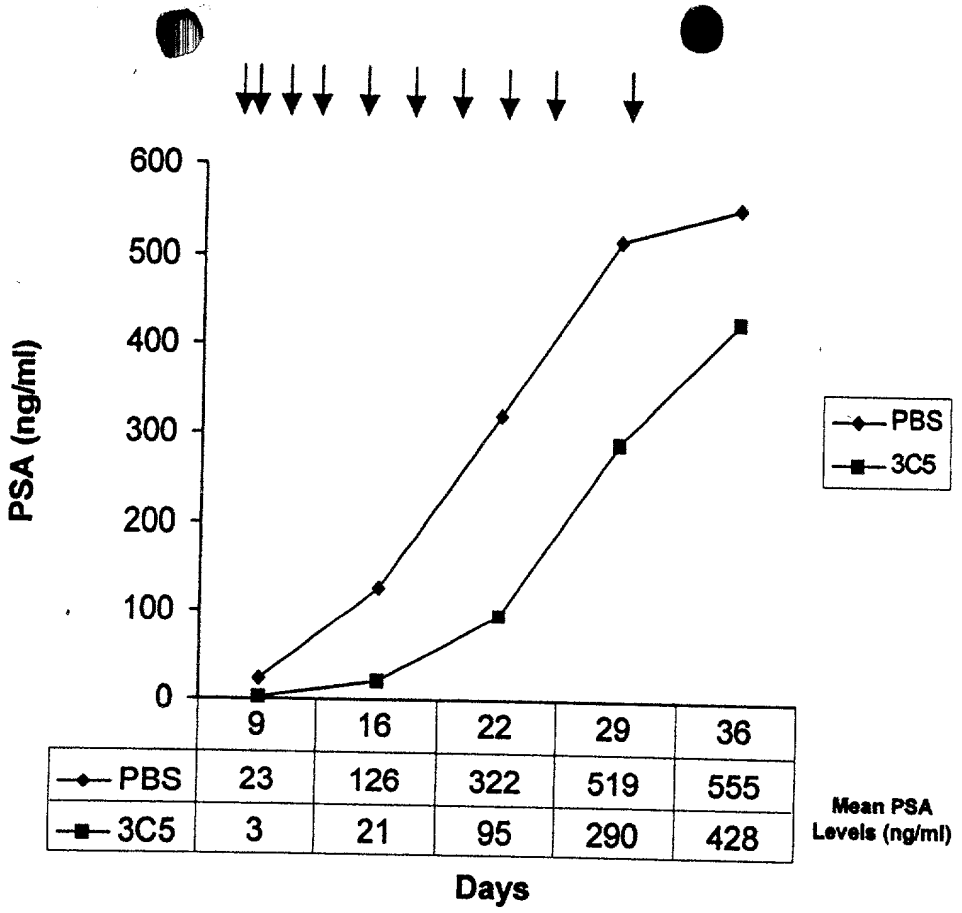

100
80
60
40
20
0
% Survival
0
20
40
60
80
100
120
Days
PBS
1G8

Figure 67

A)

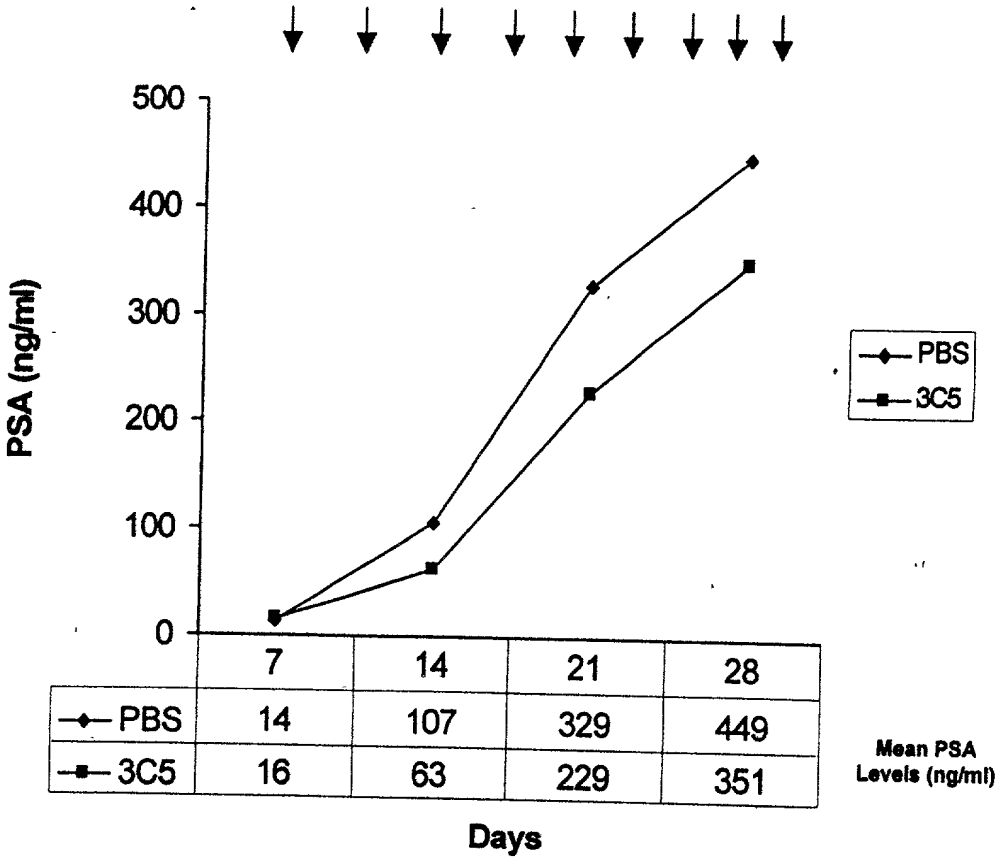


	9	16	22	29	36
PBS	23	126	322	519	555
3C5	3	21	95	290	428

Mean PSA Levels (ng/ml)

Days

B)



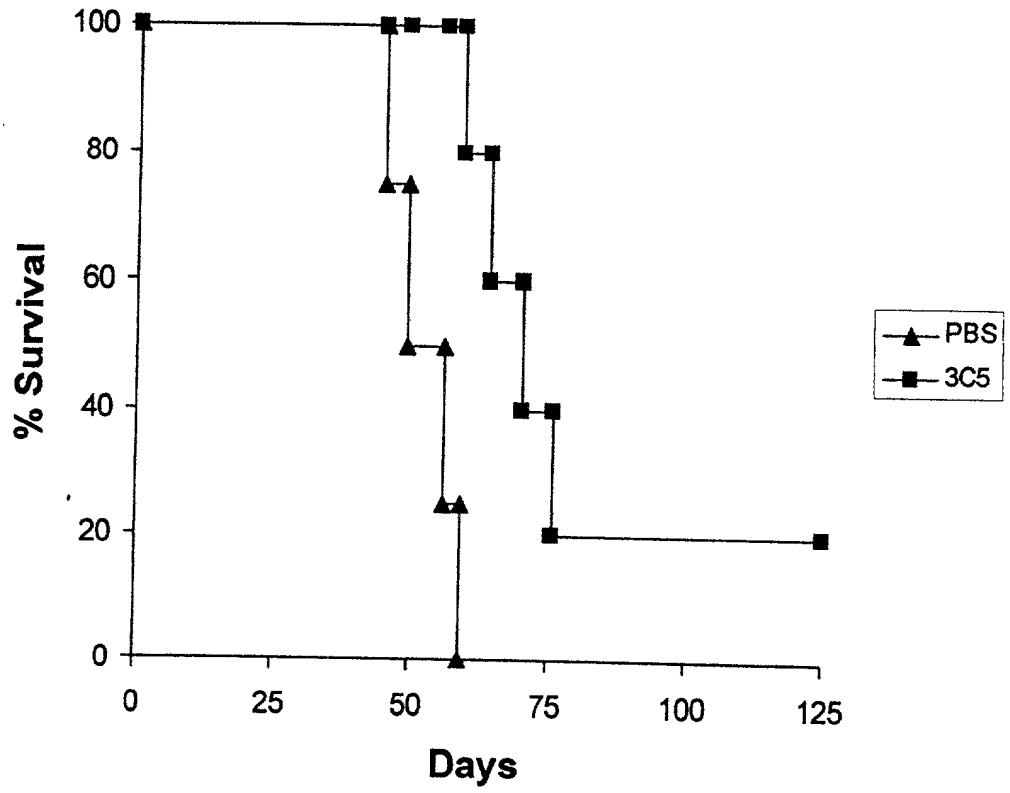
	7	14	21	28
PBS	14	107	329	449
3C5	16	63	229	351

Mean PSA Levels (ng/ml)

Days

Figure 68

A)


100
80
60
40
20
0
% Survival
0
25
50
75
100
125
Days
PBS
3C5

B)

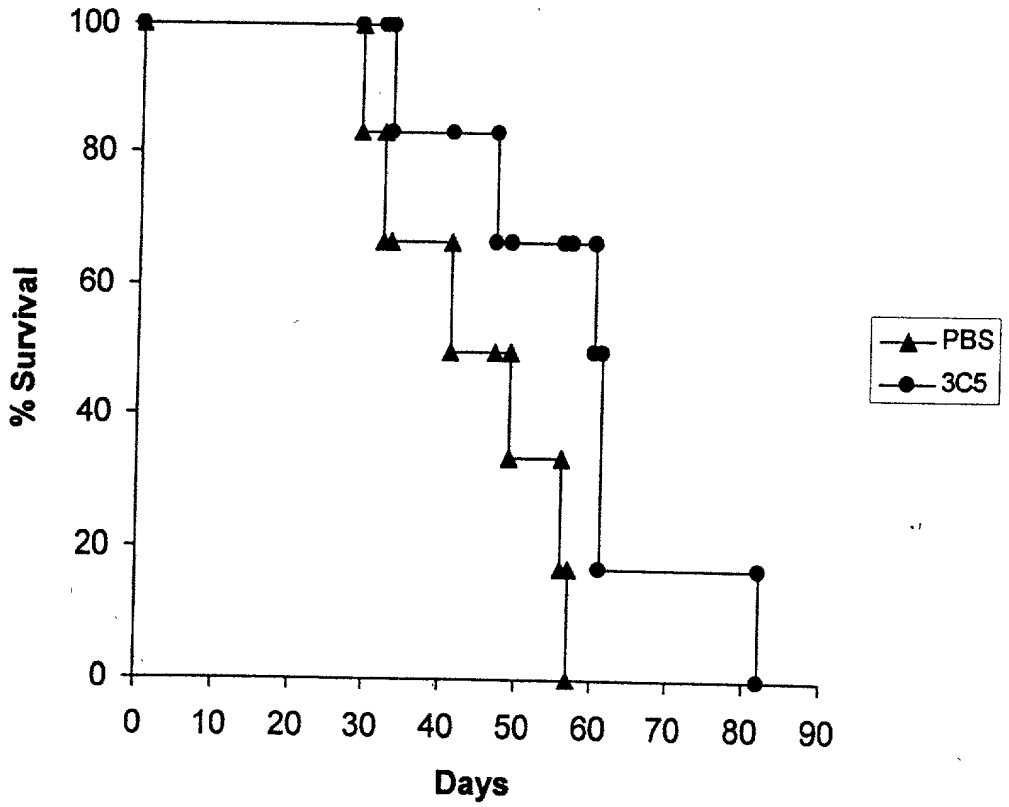
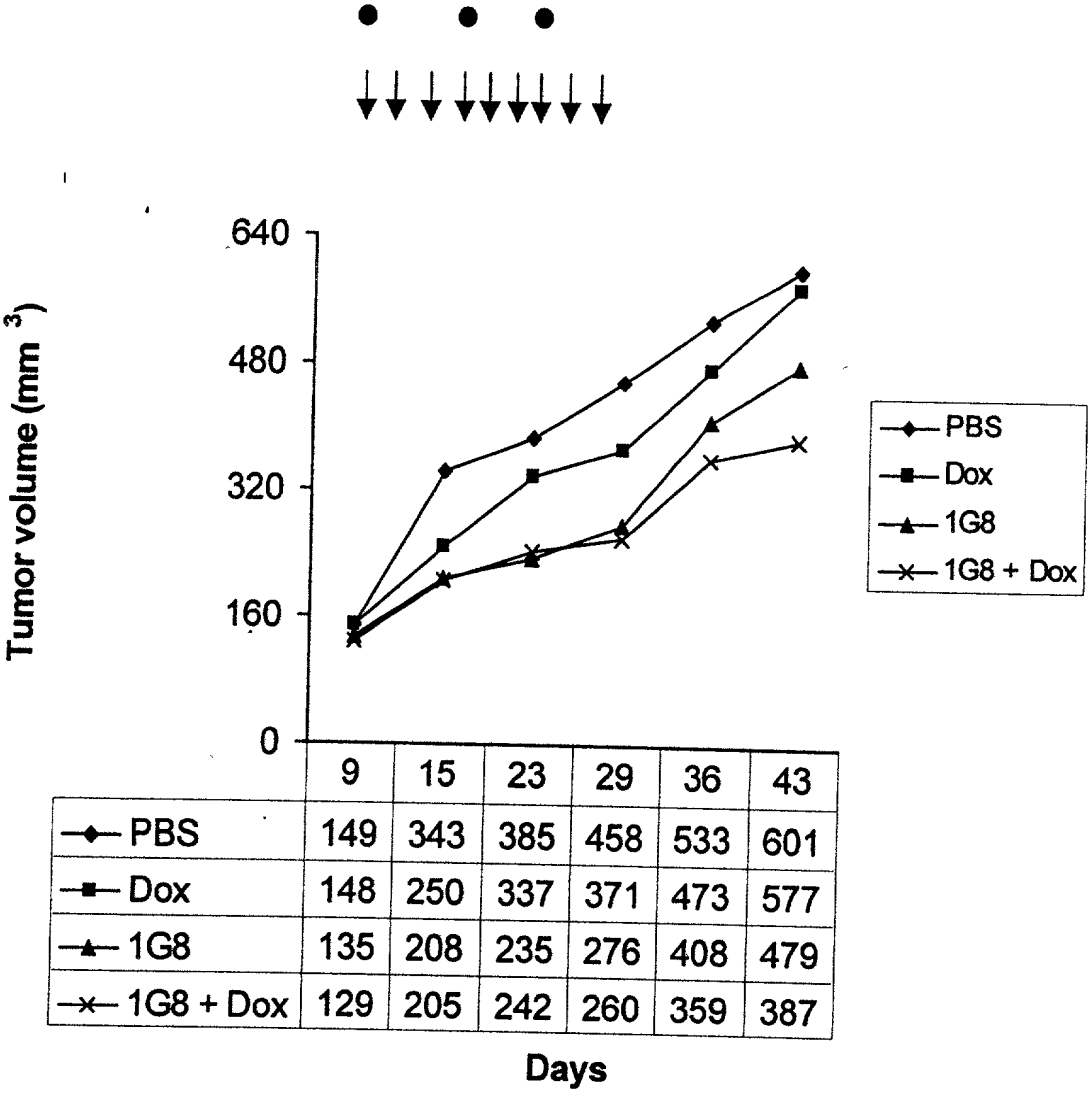

100
80
60
40
20
0
% Survival
0
10
20
30
40
50
60
70
80
90
Days
PBS
3C5

Figure 69

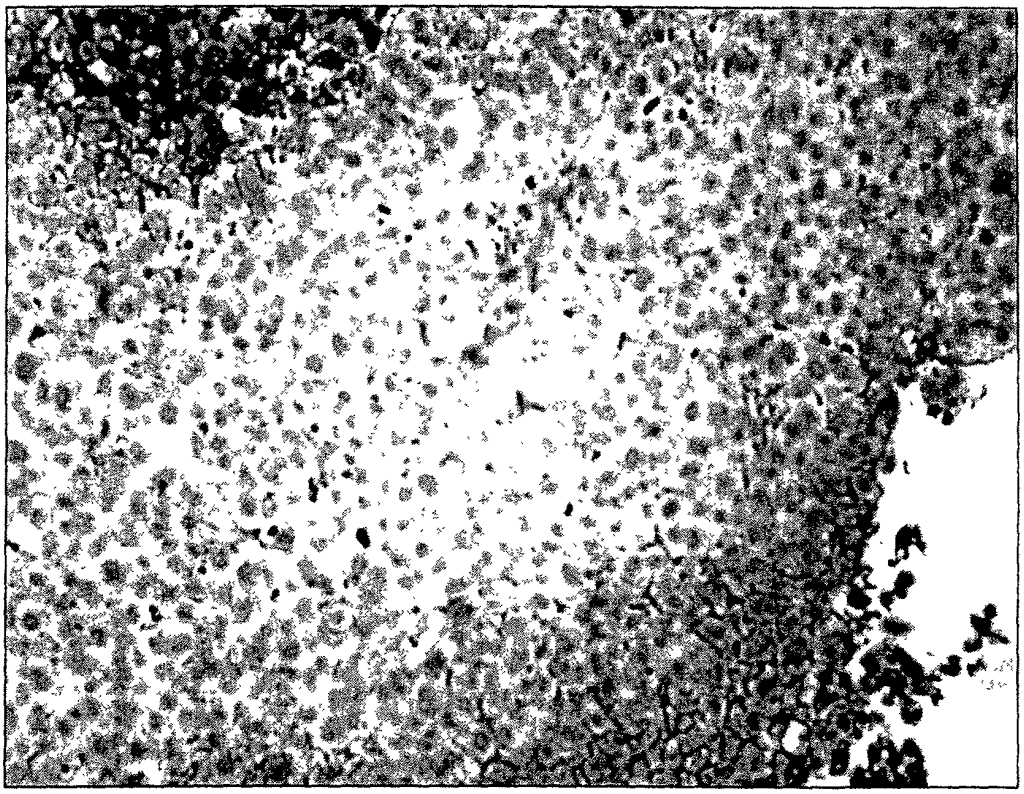


	9	15	23	29	36	43
PBS	149	343	385	458	533	601
Dox	148	250	337	371	473	577
1G8	135	208	235	276	408	479
1G8 + Dox	129	205	242	260	359	387

Figure 70

PSCA 3C5 MAb Localizes within LAPC9AD Xenograft Tissue

3C5 Treated



mIgG Treated

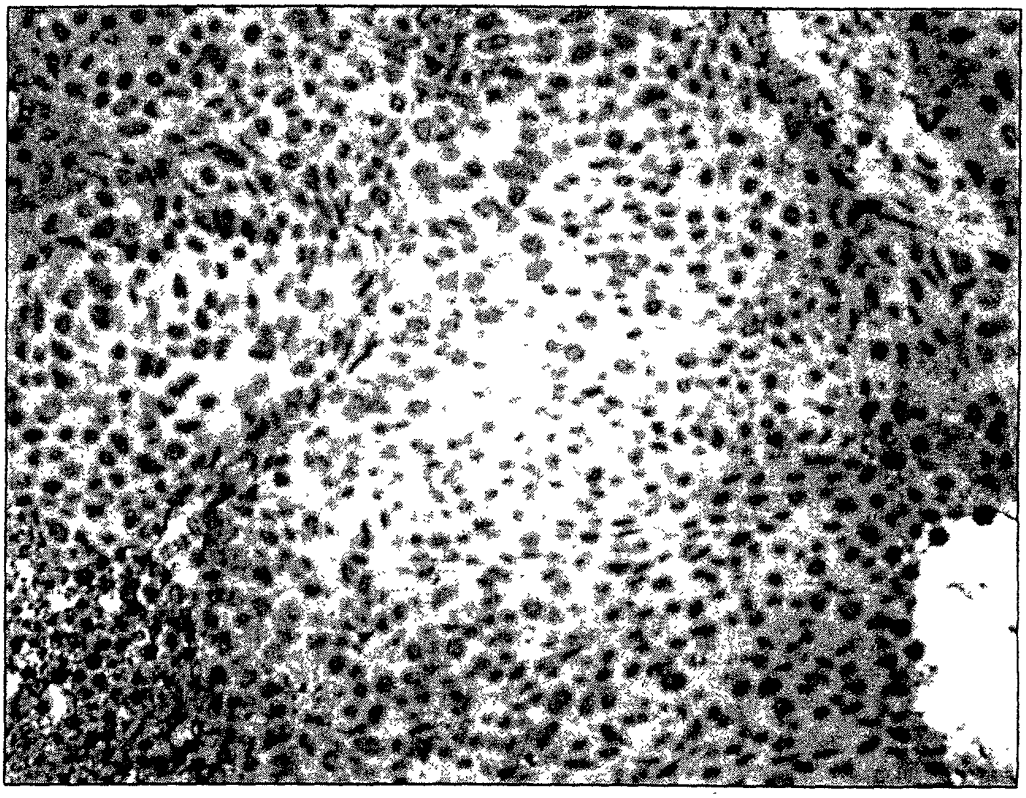
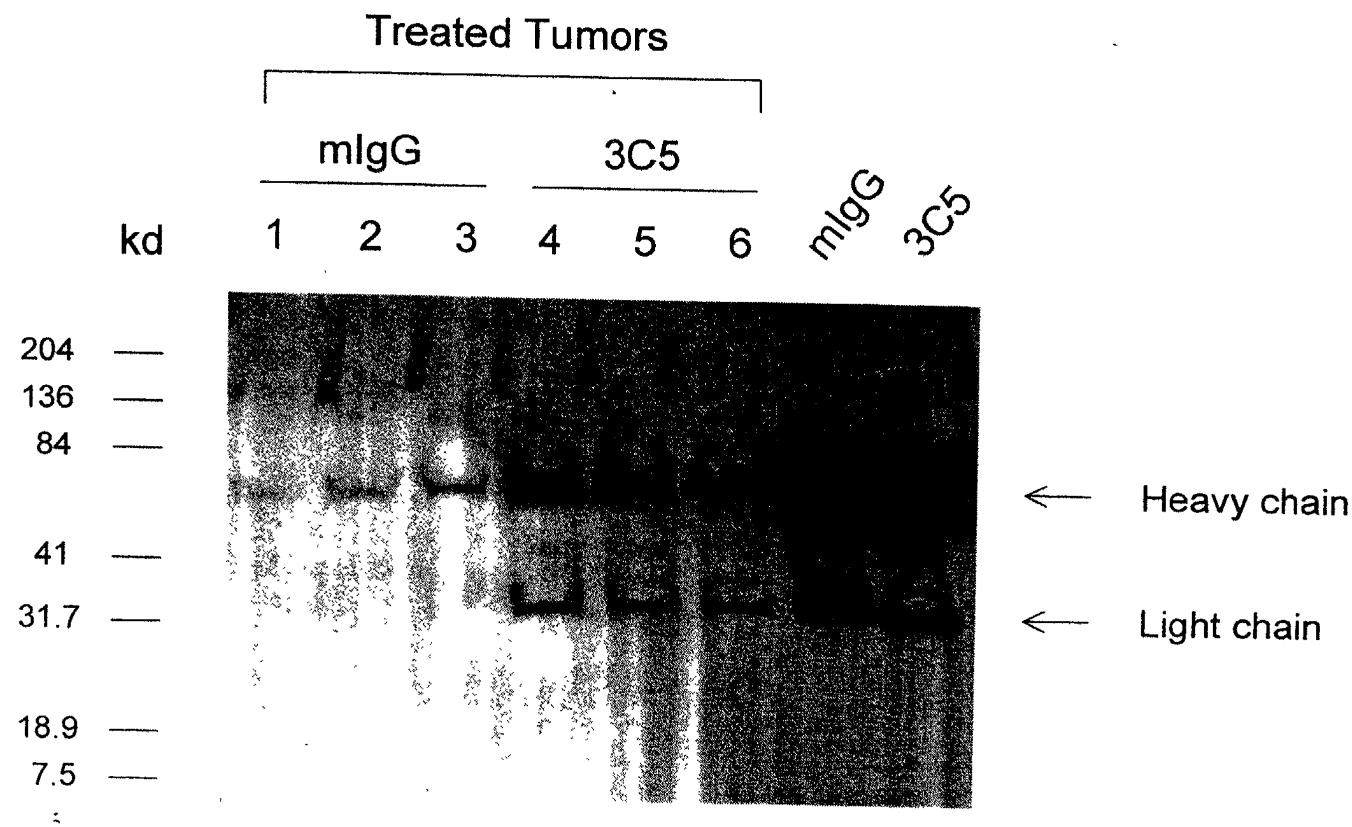


Figure 71

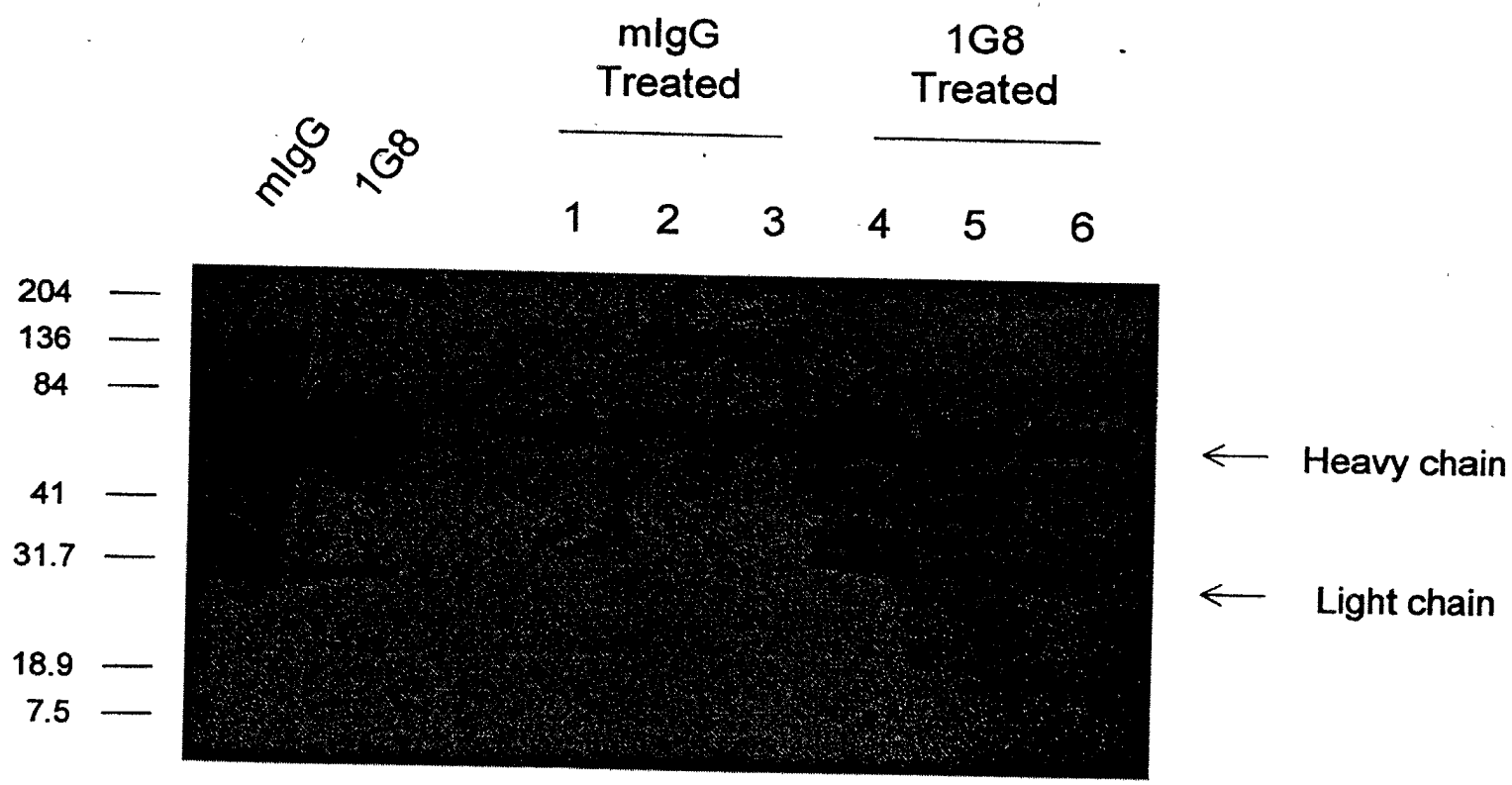
3C5 Anti-PSCA MAb is Localized to Established LAPC-9 Tumors



Western blot developed with α-mIgG/κ

Figure 72

SPECIFIC TARGETING OF THE 1G8 ANTI-PSCA MAb TO ESTABLISHED LAPC-9 TUMORS



- α-MIgG Western

Method: Mice bearing established LAPC-9 tumors (>100 mm^3) were injected with either mIgG or the anti-PSCA MAb 1G8. Tumors were harvested a week later and made into protein lysates for Western analysis.

Figure 73